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# Physiological Zoölogy

APRIL 1928

The Calcium-Potassium Ratio in Culture Media for *Planaria dorotocephala* - - - - - - - - - *Margaret R. Murray* 137

A Study of the Survival Period in the Pregnant and Lactating Cat following Bilateral Adrenal Extirpation - *Edward L. Corey* 147

The Influence of Different Parts of the Hypophysis upon Size Growth of *Rana* Tadpoles - - - - - - - *Bennet M. Allen* 153

The Effect of $CO_2$ Administration upon Parathyroid Tetany *W. W. Swingle, W. F. Wenner, and P. Stanley* 172

The Physiological Gradients in Hydra. I. Reconstitution and Budding in Relation to Length of Piece and Body Level in *Pelmatohydra oligactis* - - - - - - - - *Bernal R. Weimer* 183

The Effect of a Horizontal Temperature Gradient on the Development of the Egg of the Urodele, *Triturus torosus* *Francis G. Gilchrist* 231

The Effects of Certain Anesthetics on the Cleavage Reaction in Sea-Urchin Eggs - - - - - - *Reuben Blumenthal* 269

The Influence of the Gonads on the Plumage of Sebright Bantams *Theodore S. Eliot* 286

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# PHYSIOLOGICAL ZOÖLOGY

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# THE CALCIUM-POTASSIUM RATIO IN CULTURE MEDIA FOR PLANARIA DOROTOCEPHALA

MARGARET R. MURRAY[1]

THE object of this paper is to show that for maintaining the normal form and functions of at least certain cells of the flatworm *Planaria dorotocephala*, a higher proportion of Ca to K in the medium is required than is contained in the usual "balanced salt solutions" for vertebrate tissues. The experiments herein described arose out of previous attempts to establish a suitable medium for tissue cultures of *P. dorotocephala*.[2]

The first salt solutions tried as media for the cultures were, so far as concerns the proportions of the three essential salts, Locke and Tyrode solutions:

| Locke's Solution | | Tyrode's Solution | |
|---|---|---|---|
| NaCl | 0.8 | NaCl | 0.8 |
| $CaCl_2$ | 0.024 | $CaCl_2 6H_2O$ | 0.02 |
| KCl | 0.042 | KCl | 0.02 |
| $NaHCO_3$ | adjusted to pH 7.5 | $NaHCO_3$ | adjusted to pH 7.5 |

These solutions (having a freezing-point depression of approximately 0.5° C.) were much too concentrated for planarian tissues, as well as for the intact animals. It was necessary to dilute them to a Δ in the neighborhood of 0.20°–0.25° C.

[1] National Research Fellow in the Biological Sciences, working in the Hull Zoölogical Laboratory of the University of Chicago under the direction of Professor C. M Child.

[2] Murray (1927).

The mode of procedure in making tissue cultures of these animals was to take a number of worms of a standard size (15 mm.), which had been starved for varying periods, expose them to ultraviolet radiation for the purpose of sterilization, then place them in petri dishes containing the solutions which were to be used for culture media, cut some of them into pieces of the desired size for implantation, and leave the rest, intact, in the dishes containing the solutions. In this connection an interesting fact became apparent, which furnished the starting-point of the work recorded here.

It was found that if the salt solutions had been made up with distilled water, the intact worms which were left in them died within a few days, first having become paralyzed so that they lay on their backs or sides and when stimulated by light or shaking contracted only feebly. But if the solutions were made up with well water (which had been boiled and filtered) this phenomenon usually did not take place. In both types of solution, however, the general condition of the tissue cultures was good, and, in both, the wound contraction of the worms was affected. This matter of wound contraction had been referred to in a previous paper (Murray, 1927), where the fact was recorded that in an isotonic or somewhat hypertonic Locke solution made up with well water, the normal contraction of wounds is inhibited to a considerable extent,[1] and that this inhibition decreases as the osmotic pressure of the solution is lowered.

Tissue cultures had shown that planarian muscle cells were particularly susceptible to unfavorable conditions, among them hypo- and hypertonicity of medium. It was therefore assumed that the normal wound contraction was the result of the contact of the subepithelial muscle layer of the worm with $H_2O$, when the external epithelium was injured. But while this effect of hypotonicity doubtless operates within certain limits, the results of the present work show that the osmotic pressure of the medium is not the only factor concerned in wound contraction, and that the specific salt content of the medium is probably of greater importance.

The discrepancy between the behavior of the worms in solutions made up with distilled water and in those made up with boiled and

[1] See also J. W. Wilson (1926), Anat. Rec., XXXIV, 124, describing the absence of wound contraction in *P. maculata* placed in "isotonic" Ringer's fluid.

filtered well water was not investigated further at the time, because the only still available was a metal one, and it was thought possible that colloidal or other salts in the distillate might be the cause of the unfavorable results with the distilled water. But when a pyrex glass still was obtained and operated with a Kjeldahl bulb, the water redistilled by this means did nothing to prevent the paralysis which had been observed in salt solutions made up with water condensed in metal. The tissue cultures in the salt solutions made up with the pyrex-redistilled water remained in very good condition for some time. It was evident from these facts that the difficulty lay not with

TABLE I

| Determinations Made | Grams per Million Cc. | Hypothetical Combinations | Grams per Million Cc. |
|---|---|---|---|
| Fe | .2 | | |
| Mn | .1 | $KNO_3$ | 1.4 |
| $SiO_2$ | 7.8 | KCl | 1.1 |
| $Al_2O_3$ | 1.6 | NaCl | 7.6 |
| Ca | 49.2 | $NH_4Cl$ | .1 |
| Mg | 16.7 | $MgCl_2$ | 1.0 |
| $NH_4$ | .03 | $MgSO_4$ | 22.1 |
| Na | 3.0 | $MgCO_3$ | 41.5 |
| K | 1.1 | $CaCO_3$ | 123.0 |
| $SO_4$ | 17.7 | $SiO_2$ | 7.8 |
| $NO_3$ | .9 | $Fe_2O_3$ | .3 |
| Cl | 6.0 | $Al_2O_3$ | 1.6 |

the water used, but with the composition of the salt solution itself: either the right salts were not present, or they were not present in the proper proportions.

With this problem in mind, we consulted the analysis of the well water, in which the worms live normally for very long periods of time at the laboratory. Its components are given in Table I. The main result of boiling this water is to diminish the calcium salts to about one-half their original amount.

This analysis shows, as would be expected in this limestone region, a very high proportion of calcium salts as compared to potassium and sodium. On the basis of the analysis a series of artificial salt solutions was made up, with redistilled water, and adjusted to the suitable pH and freezing-point depression. In these, especial attention was paid to the cations and to their relative amounts; acid

and alkaline buffers were added. Two of these solutions are given below, as X–1 and X–3. In both, the intact worms behaved normally to all appearances, for several weeks, at the end of which time the experiments were discontinued. Wound contraction was not affected in these solutions. When X–1 was used as a medium for tissue cultures the protoplasm of the explants became rather stiff and viscous at first, as would be expected in the presence of so much calcium; after two to three days, however, some adaptation was evident. In X–3 tissue cultures were in excellent condition from the start.

The X–1 and X–3 solutions, though favorable, seemed needlessly complex; also it seemed desirable to come to some more exact con-

TABLE II

| | Solution X–1 (gms.) | Solution X–3 (gms.) |
|---|---|---|
| NaCl | 0.07 | 0.30 |
| $CaCl_26H_2O$ | 0.60 | 0.40 |
| KCl | 0.01 | 0.01 |
| $KNO_3$ | 0.01 | 0.01 |
| $MgCl_2$ | 0.01 | 0.10 |
| $MgSO_4$ | 0.20 | 0.02 |
| $NaHCO_3$ | 0.01 | 0.01 |
| $CaHPO_42H_2O$ | 0.01 | 0.01 |
| $H_2O$ | 100.00 | 100.00 |

clusion as to the relative amounts of calcium, potassium, and sodium which were sufficient to produce favorable and unfavorable effects upon the tissues, especially since work on contraction of the muscle cells under exposure to light was contemplated. Some research being done simultaneously in this laboratory by Miss Olga Hess on the effects of alkalis upon the respiration of *Planaria* indicated that pure potassium salts exert their general paralytic effect upon *Planaria* also. This effect was observed by the writer, too, when intact worms were placed in pure solutions of KCl, NaCl, and $CaCl_2$, respectively, which were diluted until they had a freezing-point depression of approximately 0.2° C., and raised to a pH of about 7.6 by the addition of a very small amount of $NaHCO_3$. In all three of these salt solutions, the worms began at once to contort violently; in the KCl solution the contortions soon ceased, and the worms

were completely paralyzed within one hour; they were cytolyzing within two hours. At the end of 12 hours the worms in the NaCl solution were paralyzed, and beginning to cytolyze. Within 24 hours those in the $CaCl_2$ solution were in the same condition. In NaCl and $CaCl_2$ solutions, wound contraction was approximately normal until cytolysis began. Tissue cultures accompanying these experiments with intact worms behave as follows:

In the KCl solution, the cells showed marked imbibition of water, and flame cells present in the explant were preternaturally active. Muscle cells quickly lost their normal shape, and were the first cells to cytolyze, with the possible exception of the external epithelium, which is hard to follow in the absence of individuation. The whole culture was short-lived, dying in about 36 hours.

In the NaCl solution the cells showed a tendency to produce long strands; the cells in groups appeared connected by these strands. Muscle cells tended to be relaxed and to lie in the form of long filaments. Flame cells were not conspicuous. The general condition of the culture was superior to that in KCl. Death occurred between two and three days.

In the $CaCl_2$ solution the cells at first appeared very viscous, if not actually plasmolyzed. Within three days, however, they seemed to become adapted to the situation, and had migrated widely, at the same time appearing more fluid than at first. At the end of two weeks a few muscle and pseudopodial cells were still alive.

These experiments, coupled with the behavior of the worms in the X–1 and X–3 solutions, led us to consider the possibility that while the planarian cells could tolerate large amounts of calcium, at least certain of them, i.e., external epithelium and muscle, might be affected unfavorably by very small amounts of potassium. It was primarily to test the effect of variations in the Ca:K ratio that the following experiments were divised; but it seemed necessary also to test the effect of these variations in the presence of different amounts of sodium.

Table III summarizes the results, for both intact worms, worms sectioned transversely just anterior to the pharynx, and tissue cultures taken from the same region. Four grades of wound contraction are recognized, from none at all (−), to +++, equivalent to

that in water. Similarly, four grades of paralysis are assigned, from none (−), as in water, to complete (+++). In the tissue cultures,

TABLE III

| NaCl | Experiment | | | Results | | | |
|---|---|---|---|---|---|---|---|
| | $CaCl_26H_2O$ | KCl | Solution Number | Wound Contraction | Paralysis | Individuation | Amorphous Cultures |
| 0.70 | 0.10 | 0.01 | X-28 | +++ | − | +++ | +++ |
| | 0.10 | 0.10 | X-29 | +++ | − | + | +++ |
| | 0.01 | 0.10 | X-30 | − | +++ | − | +++ |
| | 0.05 | 0.01 | X-20 | ++ | + | ++ | +++ |
| | 0.05 | 0.05 | X-21 | ++ | − | +++ | +++ |
| | 0.01 | 0.05 | X-22 | + | +++ | + | ++ |
| | 0.04 | 0.02 | X-10 | +++ | − | ++ | ++ |
| | 0.02 | 0.02 | X-12 | − | +++ | + | +++ |
| | 0.02 | 0.04 | X-11 | − | +++ | + | +++ |
| 0.50 | 0.10 | 0.01 | X-16 | +++ | − | +++ | +++ |
| | 0.10 | 0.10 | X-31 | ++ | − | ++ | +++ |
| | 0.01 | 0.10 | X-32 | + | +++ | + | + |
| | 0.05 | 0.01 | X-15 | ++ | − | +++ | +++ |
| | 0.05 | 0.05 | X-13 | + | ++ | +++ | +++ |
| | 0.01 | 0.05 | X-14 | + | +++ | +++ | ++ |
| | 0.02 | 0.01 | X-23 | +++ | − | ++ | +++ |
| | 0.02 | 0.02 | X-24 | + | ++ | + | +++ |
| | 0.01 | 0.02 | X-25 | − | +++ | + | +++ |
| 0.30 | 0.10 | 0.01 | X-18 | +++ | − | +++ | ++ |
| | 0.10 | 0.10 | X-33 | ++ | − | ++ | +++ |
| | 0.01 | 0.10 | X-34 | + | +++ | ++ | ++ |
| | 0.05 | 0.01 | X-17 | +++ | + | ++ | ++ |
| | 0.05 | 0.05 | X-26 | + | ++ | + | +++ |
| | 0.01 | 0.05 | X-27 | − | +++ | + | +++ |
| | 0.02 | 0.01 | X-35 | +++ | − | +++ | +++ |
| | 0.02 | 0.02 | X-19 | ++ | − | + | +++ |
| | 0.01 | 0.02 | X-36 | + | ++ | ++ | ++ |
| 0.10 | 0.10 | 0.01 | X-37 | +++ | − | | |
| | 0.10 | 0.10 | X-38 | ++ | ++ | | |
| | 0.01 | 0.10 | X-39 | − | +++ | | |
| | 0.05 | 0.01 | X-40 | +++ | − | | |
| | 0.05 | 0.05 | X-41 | ++ | + | | |
| | 0.01 | 0.05 | X-42 | + | ++ | | |
| | 0.02 | 0.01 | X-43 | +++ | − | | |
| | 0.02 | 0.02 | X-44 | +++ | − | | |
| | 0.01 | 0.02 | X-45 | ++ | + | | |

two points are considered: (1) the amount of individuation, which is at least partially dependent upon the condition of the external epithelium and muscle cells; (2) the general condition and survival time and persistence of cell types in the amorphous cultures. In each of these two categories four grades are recognized, as in the two preceding groups.

The chemicals were Merck's reagents. No attempt was made to recrystallize the salts in the laboratory. The figures given in the table represent only the *relative* amounts of the salts used, i.e., the grams of salt per 100 cc. constituting the basic solutions which were diluted to make the solutions in which the worms were actually immersed. Dilution was necessary, of course, in order to have the same osmotic pressure in all experiments; the freezing-point depression of each solution listed in Table III was ascertained, and the solution was diluted until its $\Delta$ was approximately 0.2° C. There had previously been added to the basic solution a small amount of a $NaHCO_3$ solution, sufficient to make the pH about 7.5 after autoclaving. Solutions X–37 to X–45, having only 0.1 per cent NaCl, were not diluted, since the $\Delta$ in all of them was considerably below 0.2° C.; for this reason also tissue cultures could not be made of them.

Table III shows consistently that where the amount by weight of the calcium salt is high as compared to the amount by weight of the potassium salt (from twice to ten times) the condition of both worms and cultures is uniformly good; where the amount of the calcium salt is equal to or less than the amount of the potassium salt, the condition of the worms and the amount of individuation in culture is fair or poor. In other words, *P. dorotocephala* requires an unusually large amount of calcium to offset potassium paralysis. The condition of the amorphous tissue cultures, however, shows not much consistent variation, but is good or very good in the majority of the solutions; nevertheless, in the solutions having the maximum of potassium salt (ten times the amount of the calcium salt) it tends to be least good.

The cells directly concerned in wound contraction, paralysis, and individuation are obviously the external epithelium, the muscle cells, and possibly, less directly, the nerve cells. The parenchyma

and endoderm, at least, may be regarded as practically inert where these functions are concerned. It is these latter types of cells which form the greater part of the explant, and hence are most conspicuous in the amorphous type of tissue culture. It is these also (particularly the parenchyma) which survive longest in solutions that are unfavorable to the intact worms. Doubtless the parenchyma cells are the most adaptable cells of the organism; according to Flexner (1898) they are the seat of regeneration at a cut end; it would seem probable also, a priori, that they are especially active in general reconstitution. Therefore it is to be expected that the parenchyma cells, and hence the amorphous type of tissue culture, should be best able to tolerate salt solutions which are unfavorable to the organism as a whole.

The constitution of the favorable salt solutions requires further comment. The low proportion of potassium salt to calcium salt (about one-half by weight, as a maximum) is evidently an essential. Beyond this, it seems possible to vary greatly the amounts of sodium and calcium, singly or together, without affecting the behavior of the worms or cultures. The amount of calcium salt may be as much as 40 times by weight the amount of potassium salt (see X–3) without unfavorable effects. From this and other data, notably Evans' (1912) report on a snail heart which beats normally in 2 per cent $CaCl_2$, it is evident that among the invertebrates at least there must exist wide variation in the use and tolerance of these three essential cations, Ca, Na, and K. Attention should be called to the fact that where the amount of sodium is low, e.g., 0.3 per cent and 0.1 per cent, a larger proportion of potassium to calcium is required to produce complete paralysis and lack of wound contraction than where the relative amount of sodium is higher. This bears out the observations of Loeb and Wasteneys (1915) that the presence of sodium favors the penetration of potassium into *Fundulus* eggs.

Table III may also throw some light on the inconsistency between the results from salt solutions made up with distilled water and those made up with well water. The latter, being rich in salts, especially calcium salts, may be sufficient to balance a small excess of potassium, such as would exist in a solution containing equal parts by weight of KCl and $CaCl_26H_2O$, and to prevent paralysis on the part of intact worms; i.e., the external epithelium may have

a slightly different threshold of susceptibility to potassium from that of muscle cells. If this is the case, then it is natural that dilution of a salt solution with the well water should tend to restore wound contraction, since it increases the proportion of Ca to K.

It should be emphasized that the calcium salt used in these experiments is the *crystalline* calcium chloride, $CaCl_2 6H_2O$, of molecular weight 219.1, instead of the "pure anhydrous $CaCl_2$," molecular weight 111, used by Ringer (1883) in his original experiments; and double the quantity of the crystalline salt *by weight* is required to provide a number of calcium ions equivalent to that in a given quantity by weight of the anhydrous salt. Therefore the minimal favorable ratio of Ca ions to K ions in these experiments is about equivalent to the ratio found by Ringer to be most favorable for the heart of cold-blooded vertebrates. Ringer's formula follows:

| | |
|---|---|
| NaCl | 0.75 |
| $CaCl_2$ | 0.0125 |
| KCl | 0.01 |
| $NaHCO_3$ | 0.1 |
| $H_2O$ | 100.0 |

Locke in 1900 used the following salt proportions for the mammalian heart, though he reports that diminishing the amount of KCl until it is only equal by weight to the amount of $CaCl_2$ was attended by no unfavorable results. This solution produces complete paralysis in *P. dorotocephala:*

| | |
|---|---|
| NaCl | 0.8 |
| KCl | 0.042 |
| $CaCl_2$ | 0.024 |
| $NaHCO_3$ | 0.02 |
| $H_2O$ | 100.00 |

Tyrode's solution (1910) used by Maximow in mammalian tissue-culture, likewise produces complete paralysis in *Planaria:*

| | |
|---|---|
| NaCl | 0.8 |
| KCl | 0.02 |
| $CaCl_2 6H_2O$ | 0.02 |
| $MgCl_2$ | 0.01 |
| $NaH_2PO_4 H_2O$ | 0.005 |
| $NaHCO_3$ | 0.1 |
| $H_2O$ | 100.0 |

The presence of the magnesium here does not compensate for the calcium deficiency so far as *P. dorotocephala* is concerned.

It has been suggested by Professor Child that this requirement on the part of *P. dorotocephala* for a relatively high Ca : K ratio in the medium may explain some of the peculiarities of the distribution of the form: it is confined so far as known to springs in limestone regions, but does not exist in many limestone springs whose superficial characteristics appear entirely similar to those of the springs in which the form is actually found.

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# A STUDY OF THE SURVIVAL PERIOD IN THE PREGNANT AND LACTATING CAT FOLLOWING BILATERAL ADRENAL EXTIRPATION[1]

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H. A. STEWART (1913) first called attention to what he considered to be an unusual prolongation of the life-span of the pregnant cat following adrenal removal. He states: "In most instances the pregnant and lactating animals survived the extirpation of the adrenal glands longer than normal, non-pregnant or male animals." However, his average prolongation in good cases of non-injected pregnant females was only 2.2 days.

In 1925, G. M. Stewart and Rogoff working with dogs cited cases of pregnant bitches which lived for considerable periods following bilateral adrenal removal. Several animals survived throughout the gestation period, and one animal gave birth to a litter of pups. These authors do not attempt to explain the reason for such prolongation of the survival period of adrenalectomized dogs, but merely suggest that possibly the corpus luteum may be involved.

In an earlier series of experiments on bilaterally adrenalectomized cats (Corey, 1926), the writer observed that pregnant individuals did not survive any longer than male or non-pregnant animals when the fact is taken into consideration that the average life-span of a double operated cat is 100 hours or more. The observations at that time were limited to only a few cases, hence no significance was attached to them. During the spring of 1927, however, the problem of the survival period of double-operated pregnant cats was carefully studied. A series of sixteen female animals was operated and their survival periods were checked against those of six operated male and

[1] A part of the expense of this investigation was defrayed by a grant from the Committee for Research in Problems of Sex of the National Research Council. Grant administered by W. W. Swingle.

non-pregnant female animals which served as controls for this particular experiment.

This problem was suggested by Dr. W. W. Swingle, of the Zoölogical Laboratory of the State University of Iowa, formerly of this laboratory, and carried out under his direction. Grateful acknowledgments are made to Dr. R. G. Harrison for laboratory facilities and the opportunity to continue the work at Yale.

All animals were operated with a 3–17 day interval between the removal of the right and the left adrenal glands. They were kept at a temperature of approximately 22° C. and were permitted the freedom of the animal room. Food was at all times available to them but no attempt was made to feed them forcibly[1] following the onset of anorexia which usually succeeds (40–48 hours) the removal of the glands. The animals were examined daily for infection but none was observed. All animals eventually died in coma, following the typical syndrome attendant upon the extirpation of both adrenals. At death each animal was carefully autopsied and histological sections were made of the ovaries in those cases in which any doubt existed as to whether an intervening ovarian cycle had taken place since abortion or resorption, where such was indicated by the condition of the uterine walls. A careful search was made at every autopsy for macroscopic pieces of cortical tissue, as is necessary in all survival experiments of this nature. No such cases have been recorded in this paper, since they are valueless for the present study.

The short survival period for animals 1, 2, and 6, as recorded in the table, is hard to explain. The fetuses found in the uterus of No. 1 (four in number) were apparently at term, or at any rate very closely approximating that stage. They were normal in all respects. The fact that the animal died tends, in the opinion of the author, to support the view of Stewart and Rogoff—that the presence of the adrenal cortex of the fetus has little or no ameliorative effect upon the symptoms of the mother.

The fetuses (four in number) found upon the autopsy of animal No. 2 were normal and somewhat over two weeks of age.

[1] Exceptions were made in the cases of Nos. 5, 6, and 19, to which 30 c.c. of milk were administered twice daily, by means of a stomach tube. No increase in the survival period due to this treatment is indicated.

Animals 3, 4, and 5 show a survival period approximately identical with that of the male control animals.

In the case of the animal in very early pregnancy (No. 7), the developing embryonic vesicle was clearly identified. The walls of the

TABLE I

| No. | Sex | Days Elapsed between First and Second Operation | Survival after Second Operation (hours) | Remarks | Survival Average (hours) |
|---|---|---|---|---|---|
| 1... | Female | 7 | 49.0 | Four term fetuses in uterus at autopsy | 86.0 (116.5 for Nos. 3, 4, and 5) |
| 2... | Female | 5 | 69.5 | Four two-week fetuses in uterus at autopsy | |
| 3... | Female | 5 | 140.0 | Four 1½-in. fetuses in uterus at autopsy | |
| 4... | Female | 8 | 110.0 | Four 1-in. fetuses in uterus at autopsy | |
| 5... | Female | 17 | 99.5 | Three fetuses in uterus at autopsy averaging 1.4 cm. in length | |
| 6... | Female | 5 | 48.0 | Five 9.0 cm. fetuses in uterus at autopsy | |
| 7... | Female | 5 | 210.0 | Very early pregnancy | |
| 8... | Female | 5 | 242.0 | Aborted four fetuses 48 hours after operation (about ½ term.) | 180.0 |
| 9... | Female | 5 | 120.0 | Aborted three 2-in. fetuses 36 hours after operation | |
| 10... | Female | 6 | 97.0 | Gave birth to three kittens 16 hours after operation. Suckled young for 24 hours | |
| 11... | Female | 7 | 69.5 | Gave birth to four kittens 8 days before final operation. Suckled young 24 hours, four corpora lutea on ovaries | |
| 12... | Female | 7 | 216.0 | Resorption of three fetuses | |
| 13... | Female | 7 | 141.0 | Non-pregnant | 110.0 |
| 14... | Female | 5 | 71.0 | Non-pregnant | |
| 15... | Female | 7 | 119.0 | Non-pregnant | |
| 16... | Male | 5 | 95.0 | Control | 127.3 |
| 17... | Male | 5 | 118.0 | Control | |
| 18... | Male | 5 | 169.0 | Control | |
| 19... | Female | 3 | 42.0 | Pregnant (?) Four corpora lutea found on ovary at autopsy | |

uterus were considerable distended and the placental formation normal.

Animal No. 8 aborted four fetuses 48 hours after the second operation. They were apparently at about half-term. (Since in no case was the time of insemination recorded, the age of all fetuses is necessarily a rough estimate.) Upon autopsy, 194 hours later, one almost completely resorbed fetus was found in the uterus. The walls of this organ were distended, the remains of the placenta were of a typical dark-brown coloration, and the uterine vessels were greatly enlarged.

Animal No. 9 aborted three two-inch fetuses 36 hours following the second operation. It, however, lived for 120 hours after the second operation, dying with typical symptoms of adrenal insufficiency.

No. 10 gave birth to three full-term kittens 16 hours after the second operation. The young were suckled for 24 hours, after which the mother paid them no further notice. She died 97 hours after the removal of the second gland. At autopsy the uterus was found to be empty.

No. 11 gave birth to four kittens (two dead) eight days before the removal of the second gland. The kittens were suckled for approximately 24 hours following complete epinephrectomy. The mother died 69.5 hours following the second operation.

Animal No. 12 showed practically complete resorption of three fetuses. The condition of the uterus indicated quite clearly that no ovarian cycle had succeeded the pregnancy. The short time-interval from operation until death makes a case of complete resorption somewhat dubious. It can only be looked upon as a case of very early vesicle formation, followed by a succeeding rapid resorption. The walls of the uterus were quite markedly distended and the placenta of a characteristic brownish color. The uterine vessels were greatly distended.

The non-pregnant female animals were employed as control animals, their survival period being approximately identical with that of the male controls. As pointed out in a previous paper (Corey, 1927), a survival period of approximately 100 hours, or over, must be looked upon as the normal life-span for bilaterally epinephrectomized

cats. However, if fluids are forcibly fed or are injected, the life-span is considerably prolonged (Corey, 1926.)

Animal No. 19 is a case of doubtful early pregnancy. No fetuses were found in the uterus at the time of autopsy, but four corpora lutea were clearly made out on the surface of the ovaries. The uterine vessels were somewhat distended.

As will be seen from the accompanying table, the pregnant animals (with the exception of Nos. 7, 8, and 12) show a survival period closely approximating that of the control animals. The fact that none of the pregnant animals (with the exception of No. 10) lived until the delivery of its litter indicates that whatever mechanism may be present in the dog which enables the epinephrectomized mother to live until the culmination of the pregnancy, this mechanism is apparently lacking in the cat. Since No. 10 delivered its litter only 16 hours after the second operation, it can hardly be taken as a case controverting the foregoing statement.

Animals 7, 8, and 12 survived considerably longer than the average period for adrenalectomized cats but not longer than double-operated cats treated by forcing fluids. As shown in the table, animals 7 and 12 were cases of very early pregnancy, whereas No. 8 was a case of advanced pregnancy. The longest survival period for a pregnant animal was 242 hours, whereas the longest survival period for a non-pregnant cat was 169 hours. The writer is not inclined to lay too much stress upon these cases since they all died within 10 days following the second operation.

It is interesting to note that cats and the dogs react differently to adrenal insufficiency in several respects. For example, adrenalectomized dogs usually show gastro-intestinal disturbances such as vomiting; they also are subject to yelling fits and hallucinations, and upon autopsy show great congestion and extravasation of blood into the gastro-intestinal tract. Operated cats do not present these symptoms, or if so are exceptions to the general rule. So, too, with regard to the effect of pregnancy and lactation upon the survival period following adrenal removal. In the dog the life-span following adrenalectomy is apparently greatly prolonged by pregnancy (Stewart, G. M., and Rogoff), but such appears not to be the case with cats.

## CONCLUSIONS

1. The presence of the adrenal cortex of the fetal cat has little or no ameliorative effect upon the syndrome of the epinephrectomized mother.

2. The survival period of bilaterally epinephrectomized pregnant cats is approximately the same as that of male and non-pregnant female animals, similarly operated.

3. Whatever mechanism is present in the dog enabling the epinephrectomized mother to live until the culmination of the pregnancy, this mechanism is apparently lacking in the cat.

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# THE INFLUENCE OF DIFFERENT PARTS OF THE HYPOPHYSIS UPON SIZE GROWTH OF RANA TADPOLES[1]

(Ten figures)

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IT IS quite generally conceded that the hypophysis exerts an influence upon size growth. The earlier papers along this line and the clinical cases that point in this direction are reviewed in all of our textbooks, so it is worth our while in this paper to review briefly only those papers that have a more direct bearing upon the question in hand.

The study of this problem may be made by extirpation, by feeding, and by implantation. In the present paper, we shall consider the growth effects produced by implanting the anterior, intermediate, and posterior lobes of the hypophysis of adult frogs into hypophysectomized and thyroidectomized tadpoles. No one has yet succeeded in making implants of the pars tuberalis. This would be difficult but should not be impossible. It is certainly important. An account of an exploratory study of this subject has already been given (Allen, 1920). In the meantime, other phases of the results of gland implantation have been considered and it was not until 1925 and 1927 that the phase of the work here presented was undertaken on a sufficiently large scale and with accurate quantitative determinations.

Statements of the writer (Allen, 1918), regarding the influence of the thyroid gland and hypophysis upon growth in size, were made rather guardedly and have dealt with measurements of preserved specimens, no effort being made to follow individuals in their growth from week to week. The conclusion drawn was that the tadpoles are distinctly retarded in their growth as a result of hypophysectomy, while removal of the thyroid gland does not retard growth in size,

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but on the other hand, because its presence is essential to metamorphosis, its removal permits the tadpole to continue growing beyond the period when metamorphosis causes disappearance of the tail and shrinkage of the body in normal controls. Because of this fact, thyroidectomized tadpoles ultimately grow to a size far beyond that reached by normal tadpoles. This is sometimes true of hypophysectomized tadpoles but not to the same degree.

Hoskins and Hoskins (1919) state, "the thyroidless animals (tadpoles) grow more rapidly than the controls but the difference is not very great until near the time of metamorphosis. When the controls at this period have nearly reached their maximum length their growth seems to slacken and their length does not seem to change for a short period of time before it starts to decrease during metamorphosis," Hoskins and Hoskins considered the enlargements of the anterior lobe of the hypophysis to play a part in causing greater growth of the thyroidectomized tadpoles.

Smith (1920) carried on an extensive series of experiments upon the influence exerted upon the size growth of *Rana boyeli* and *Bufo boreas* by feeding the anterior lobe of the beef hypophysis. Hypophysectomized tadpoles on liver diet showed a decided retardation in growth when compared with normal controls kept upon the same diet. A third set fed with fresh anterior lobe of the hypophysis underwent growth in size almost identical with that of the normal controls except for the fact that they continued growth in length after the controls had begun to undergo the shrinkage involved in metamorphosis. The rate of growth up to normalcy was proportional to the frequency with which anterior hypophysis was fed.

Smith's work upon thyroidectomized tadpoles is best summed up in his own words (p. 67):

> Observations, however, made on two groups of thyroidectomized frog larvae, one group (seventy-two specimens) fed on anterior lobe, the other (fifty-nine specimens) fed on liver show that their growth is identical in rate with that of their normal brothers or with albinos (hypophysectomized specimens) supplied with a continuous diet of the fresh anterior lobe. Their size is never attained by a more rapid growth rate, but rather, as in hypophysectomized tadpoles fed with anterior lobe material, by a prolongation of the growth period. . . . . The enlarged pituitary which these animals possess does not appear to have supplied any growth stimulus in excess of the normal.

On the other hand, in the case of *Bufo* tadpoles he found that the thyroidless tadpoles fed with a liver diet grew much more rapidly than the hypophysectomized tadpoles fed anterior hypophysis.

Swingle (1921) made heteroplastic intraperitoneal implants of the anterior lobe of the hypophysis of adult *Rana catesbiana*, *Rana pipiens*, and *Rana clamitans* into normal immature larvae of *Rana catesbiana*. This induced a tendency toward metamorphosis but did not cause an appreciable growth in size. Swingle (1921) also made homoplastic implants of the anterior lobe of the hypophysis that resulted in definite size growth.

Evans and Long (1922) produced gigantism in the rat by repeated intra-peritoneal injection of extracts of the anterior lobe of the hypophysis of cattle.

Uhlenhuth (1922 and 1923) produced marked growth in *Ambystoma tigrinum* by feeding the anterior lobe of the hypophysis causing them to become 25 per cent longer than the longest-known normal specimen. Liver feeding produced a far less marked growth acceleration.

P. E. Smith and J. P. Smith (1923) produced growth acceleration in the development of hypophysectomized tadpoles of *Rana boylei* by administering intra-peritoneal injections of the anterior lobe of the beef hypophysis three times weekly. These tadpoles reached a volume three times that of the non-injected specimens. Normal tadpoles were made to develop to twice the volume of their controls. An even greater increase in the volume of thyroidless tadpoles was produced by these intra-peritoneal injections. The same method was applied to a study of the influence of the intermediate lobe of beef hypophysis but this did not result in any decisive growth response.

Allen (1925) gave a preliminary account of the experiments that form a basis for this paper so far as they apply to the influence of the implanted anterior, intermediate, and posterior lobes of the hypophysis upon the growth of hypophysectomized tadpoles. The growth of these was compared with that of normal and hypophysectomized tadpoles without implants.

Křížnecký (1924) fed "pars infundibularis and pars glandularis" of the hypophysis separately to tadpoles and came to the conclusion that the growth stimulating influence of the pars infundibularis is

transformed to a growth-inhibiting influence by mixing it with plant food and that a growth-inhibiting influence of the pure pars glandularis is changed to growth stimulating influence by addition of plant elements to the food. This view was greatly modified in a later paper, Křížnecký and Podhradský (1926), according to which they still find that a mixed diet of glandular material and plant food produces more active growth than the unmixed glandular substance; but they come to the conclusion that *both* the pars infundibularis and pars glandularis induce more rapid growth than attained by feeding lean veal.

## MATERIAL AND METHODS

As stated above, the experiments described in this paper were performed during 1925 and 1927 upon *Rana aurora draytoni*, all the tadpoles used during a season being reared from one clump of eggs. About five hundred tadpoles were operated for the removal of the buccal anlage of the hypophysis and an equal number for the removal of the thyroid gland. Only the best of these were used in the experiments. Measurements were made of normal controls, hypophysectomized and thyroidectomized tadpoles without implants, and of the latter two classes with implants of the anterior, intermediate, and posterior lobes severally of the adult hypophysis. One hundred thirty-five implants were made in 1925 and one hundred seven in 1927. For one reason or another, many of these were not usable. None were, however, eliminated from consideration without obviously good reason—early death, disease, failure in gland removal, or in implantation.

The operation for removal of these glands is made in very early stages when their anlagen first develop and is performed according to the technique worked out by the writer and described (Allen, 1916 and 1917).

The material for implantation is taken from adult frogs, the animals being paralyzed by cutting across the cranial end of the spinal cord with bone forceps. Instruments sterilized in 95 per cent alcohol are used to extract the brain, the hypophysis being usually found adherent to the floor of the brain case from which it is removed with sterilized forceps and placed in boiled normal salt solution in a sterilized Syracuse dish. The lobes of the gland are dissected apart under

a binocular microscope. In this operation, two needles, one an ordinary one and the other sharpened to a cutting edge, are used. The anterior lobe of the hypophysis can be quite readily separated from the others, but intermediate and posterior lobes are so closely adherent to one another that it is necessary to cut them apart at a slight distance on each side of the plane of junction in order to secure pure gland material of each uncontaminated with that of the other. The anterior lobe of the hypophysis is cut in two and only one-half used in each implantation. In the case of the intermediate and posterior lobes, the entire piece is used. This is done because of the smaller initial size of these portions, and because of the fact that about one-half of each is wasted in observing the precautions of separation as stated above.

The implantation is made by cutting a slit in the skin on top of the head just median to the right eye and working a pocket in the subcutaneous tissue. The implant is shoved into this space with as little loss and injury as possible and with a minimum of contamination by contact with the surface of the skin. At best, dependence must be placed upon the resistance of the tissues to infection and in this we are not disappointed, because a check made months later shows the implant in the great majority of cases to be in a healthy condition. A study is being made of the changes, if any, that have occurred in the implant; but it is not essential to acceptance of the results included in this paper. Success in this operation is demonstrated by the marked acceleration of growth caused by implantation of the anterior lobe of the hypophysis into hypophysectomized tadpoles, and by the striking color changes (Allen, 1920), produced by implantation of the intermediate lobe. This is fully as small as the implanted material of the posterior lobe, and much smaller than the half-anterior lobe implants. Much of it is often lost because of its soft consistency. In this regard, it offers quite a contrast to the other parts involved in the experiments. In spite of these facts, it causes a most intense color change, tadpoles implanted with it assuming a black color far deeper than that of the controls. This feature is mentioned here as further evidence that the technique followed is effective.

Measurements were made each week for from eight to fifteen

weeks. Two methods were used. In 1925, the procedure was to anaesthetize the tadpole by placing it in water shaken up with a small amount of ether. Each was then put in a small narrow dish with a glass bottom on which a scale ruled to half millimeters was cemented. The anterior tip of the head was placed against a vertical wall and a reading made to determine the total length of the tadpole to the tip of the tail. The reading was made to tenths of millimeters by estimation, the observation being taken by use of the low powers of a binocular microscope. This method proved pretty accurate, as shown by frequent repetitions of the determination. Some specimens were killed by the anaesthetic—a decided misfortune after measurements had been taken for several weeks, because the specimen had to be disregarded if length determinations did not extend through the entire period. Slight initial size differences and differences in vigor would cause the results to be seriously vitiated if any such incomplete cases were used in computing the final averages.

The tadpoles were kept singly in half-gallon butter crocks that were cleaned each week. The food was administered uniformly, being renewed every alternate day. A standard ration was prepared, consisting of five parts wheat flour, one part powdered alfalfa leaves, and one part egg yolk. This was mixed into a paste with a little water dried in a thin layer, and fed in broken flakes. The ration was varied from time to time by feeding fresh liver and the yolk of hard-boiled egg. Living algae were kept in the crocks and they were given plenty of indirect light that was uniformly distributed. In the 1927 cultures an increase in growth rate was caused by the uniform introduction of fresh algae during the course of the experiment. This is seen in the graphs. It has little significance, however, because it affected all classes alike.

In the 1927 experiments, the length determinations were made by using a Leitz Macroscopic Drawing Apparatus with a magnification of $\times 4$ during early stages and $\times 3$ during the later part of the determinations. Horizontal lines were drawn on a sheet of paper placed in the position where the image of the tadpole is projected by the prism. With care to insure steadiness of the observer, points were marked on a line to indicate the cranial and caudal tips of the body and each line labeled with the number of the tadpole measured

on it. These were later measured with a ruler and the actual size computed. This method causes a minimum of injury to the tadpole because anaesthesia is not necessary, although the task of inducing the tadpole to remain quiet, straight, and erect at the proper place is a very severe strain on the patience of the observer. This is not as accurate as the method employed in 1925, there being a possible error of one-half millimeter in the observation. However, each determination was several times repeated and the method was used consistently on all classes of experiments. This, together with the number of tadpoles used, served to give reliable averages. Furthermore, the results are in harmony with the determinations made by the other method described above.

Anyone who has worked extensively with tadpoles has observed the decided individual differences in vigor and growth rate. These are fairly well marked by the time a length of 20 to 25 mm. has been reached. Tadpoles of comparable size were selected to obviate, so far as possible, the factor of the individual difference. Since the removal of the hypophysis and thyroid anlagen takes place before the tadpoles have reached a length of 7 mm. (60–120 days before the commencement of measurements), it is quite clear that specimens showing general defects resulting from the operation are readily eliminated.

The removal of the buccal anlage of the hypophysis prevents the development of the anterior, intermediate, and pars tuberalis portions of that gland. Both Smith (1920) and Allen (1918) agree that the posterior lobe—pars nervosa—develops to a certain degree; Smith claims that it does not reach normal size.

The 1925 specimens were dissected to check the success of the extirpation operation and the few cases where there had been failure to remove the gland were discarded. Success in the removal of the buccal anlage of the hypophysis results in conspicuous pigmentary changes, while failure in this leaves the tadpole with its normal color, except in a few cases where the gland is very small. Successful removal of the thyroid gland or of the hypophysis prevents the growth of the hind limb buds beyond the earliest stage. Implantations are made after the differences in limb growth between the normal controls and operated tadploes have begun to appear. In

certain cases where there were small remnants of the thyroid gland or hypophysis, subsequent limb growth served to indicate its presence, and these cases were eliminated from the final computations.

## RESULTS

The figures in parentheses on certain graphs show the number of specimens included in the average represented by the curve so indicated. In certain other curves where no number in parentheses is given, the growth of a single specimen is represented. Some of

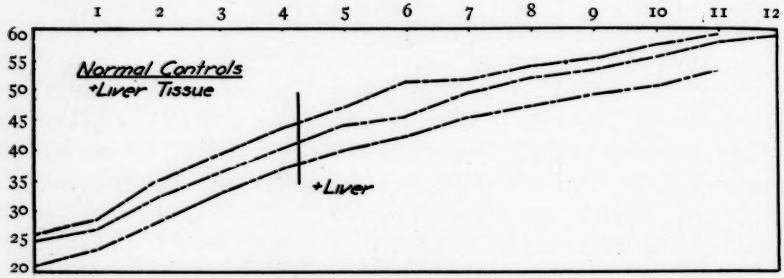


FIG. 1.—Graphs showing the growth of normal *Rana aurora draytoni* tadpole controls into which liver implants were made just after the fourth week to determine whether the operation alone might have a stimulating effect on growth. Ordinates indicate total body length in millimeters and abscissae mark time from the beginning of the experiment measured in weeks.

these latter showing change in the curve resulting from anterior hypophysis implants are most instructive.

The objection might be raised that the growth increases recorded are due to the nutritive influence of the implant. In order to test this question, implants of liver substance were made into three controls and were found to produce no growth effect in any of them (Fig. 1),[1] in spite of the fact that the liver material was greater in amount than any of the hypophysis implants. The next point that might be raised would concern the question whether the effects are produced by *absorption* of hormones from the implants and not by their functional activity. The test of this was made by killing

[1] For the explanation of this and the following graphs see the explanation of graphs following the text.

the implants in certain cases by immersion in 2 per cent formalin solution, after which they were thoroughly washed in boiled water. In other cases, glands were boiled before implantation. In all cases these experiments were controlled by killing only one half of the anterior lobe of the hypophysis and implanting the other half in a living condition into a tadpole of the same lot and of comparable size. The graphs illustrating the results of these experiments are shown in Figures 2 and 3. In all cases, it is seen that the implantation of dead anterior hypophysis material does not influence growth while living material of this gland produces definite acceleration of growth.

The points discussed above serve to give us a basis for evaluating the results that we shall now proceed to discuss. They show that the results of gland implantation outlined below are not due to the introduction of non-specific living tissue nor to the absorption of hormones from inactive implanted tissue, but instead they are caused by the active functioning of the endocrine glands involved.

A few points regarding the interrelations of the anterior lobe of the hypophysis and the thyroid gland should be kept in mind in considering these gland implantations. The removal of the hypophysis prevents the development of the thyroid gland and the accumulation of any considerable amount of colloid. The thyroid gland can be brought back to normal development and function by implantation of the anterior lobe of the hypophysis (Allen, 1920 and 1927). This results in a tendency to normal metamorphosis which in later stages of experiment brings about the shortening of the tail. On the other hand, removal of the thyroid gland causes the anterior lobe of the hypophysis to develop to abnormal size as shown by Rogers (1918), Hoskins (1919), Larson (1926), and others. It will thus seem that the thyroidectomized tadpoles would have at least a normal amount of anterior hypophysis secretion, probably an excess amount, depending upon the findings of histological examination.

In the work under consideration, the graphs (Fig. 4) show that thyroidectomized tadpoles grow at substantially the same rate as the normal controls. This is an interesting point somewhat contrary to expectation, because the larger size of the anterior lobe of the hypophysis in thyroidectomized specimens would lead one to ex-

pect increased growth. Hypophysectomy, on the other hand, causes a decided retardation in size growth (Fig. 4).

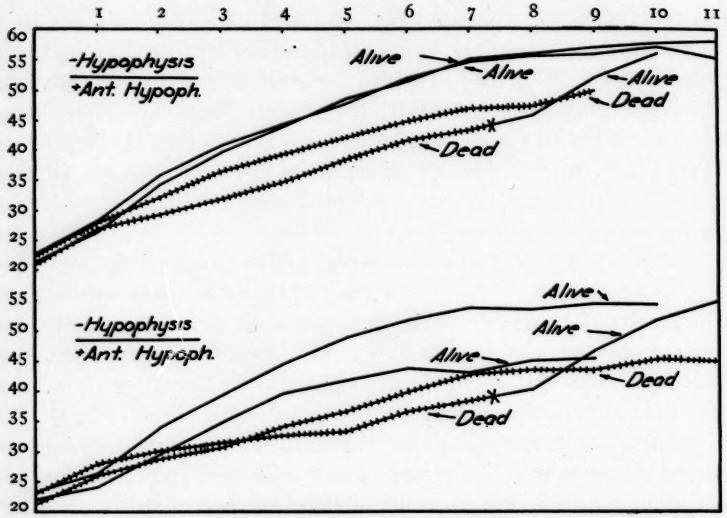


FIG. 2.—Graphs showing growth of hypophysectomized *Rana aurora draytoni* tadpoles into which implants of the anterior lobe of the hypophysis had been made to determine whether effects produced are the result of resorption or of functional activity of the implant, the condition of the implant being indicated by the words "alive" or "dead." Ordinates indicate total body length in millimeters and abscissae mark the time from the beginning of the experiment measured in weeks.

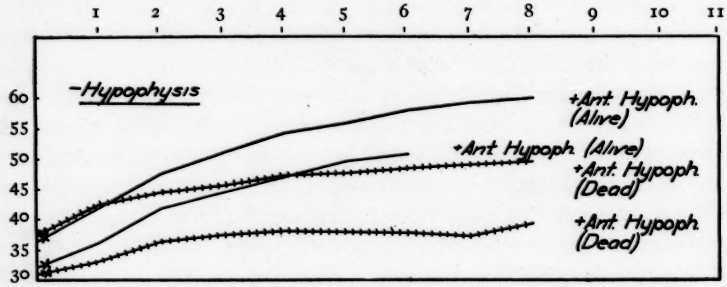


FIG. 3.—Graph showing the same points as in Fig. 2

The implantation experiments which we shall next consider afford a crucial test of the relative influence upon size growth exerted by the posterior, intermediate, and anterior lobes of the hypophysis. Extirpation of the buccal anlage of the hypophysis results in the complete absence of the anterior and intermediate lobes, while ac-

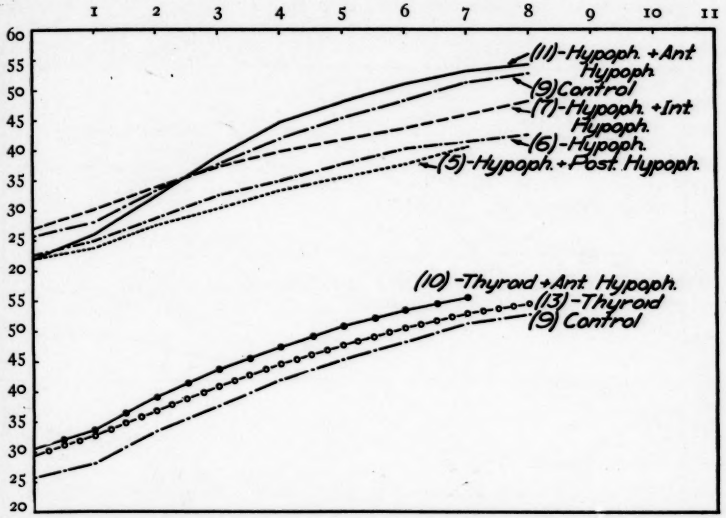


FIG. 4.—Graphs showing the rate of growth of tadpoles of *Rana aurora draytoni* studied in 1925. The numbers in parentheses indicate the number of specimens averaged in the group indicated. The abbreviations are self-evident and have been explained in Explanation of Graphs. Controls are normal tadpoles and the line indicating their growth is repeated in the upper and lower group. Ordinates indicate total body length in millimeters and abscissae mark the time from the beginning of the experiment measured in weeks.

cording to Smith (1920) the absence of these structures causes a marked retardation in the development of the posterior lobe (pars nervosa). Even though this be the case, implantation of the posterior lobe of the adult will afford a great excess of secretion of that gland. This is demonstrated by certain effects that are not treated in this paper. Implantation of the intermediate lobe is effective in producing striking color changes. On the other hand, neither of these portions of the hypophysis complex has the slightest tendency to re-

store the rate of *growth* to normal. The graphs in Figure 4 show that hypophysectomized tadpoles into which one or the other of these two lobes (intermediate and posterior) has been implanted show no change in rate of growth.

Implantation of the anterior lobe of the hypophysis into hypophysectomized tadpoles on the other hand causes decided acceler-

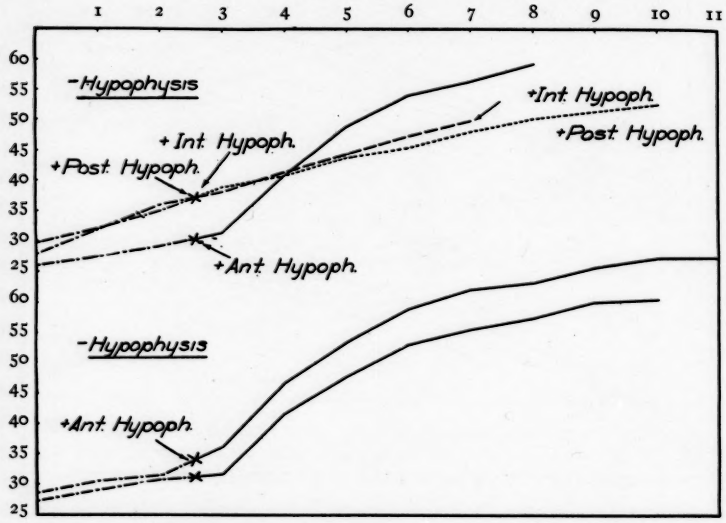


FIG. 5.—Graphs showing growth of hypophysectomized tadpoles of *Rana aurora draytoni*. At the time marked by *X*, implants of different lobes of the hypophysis were made as indicated by the abbreviations. The glandular material implanted was in each case taken from the same adult frog. Ordinates indicate total body length in millimeters and abscissae mark the time from the beginning of the experiment measured in weeks.

ation in growth as seen in Figure 4. The hypophysectomized tadpoles had lagged far behind the normal ones in growth, being on the average about 4 mm. shorter at the time when the implants were made. Those tadpoles into which the anterior lobe of the hypophysis was implanted grew very rapidly, exceeding the normal controls in less than three weeks and maintaining their lead throughout the eight weeks of the experiment.

While thyroidless tadpoles have a well-developed hypophysis in

which the anterior lobe shows actual hypertrophy, growth in length is in no wise affected, as stated above. What would happen if we greatly increase the amount of material of the anterior lobe of the hypophysis by implanting one-half of an adult gland, fully ten times

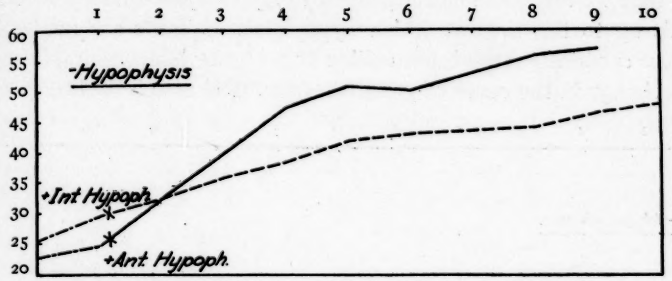


FIG. 6.—Graphs showing the growth of hypophysectomized tadpoles of *Rana aurora draytoni* as in Fig. 5.

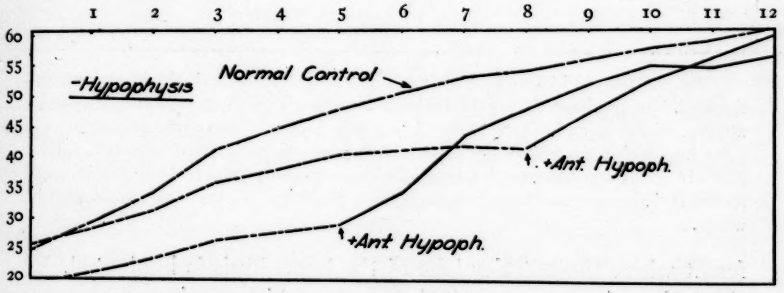


FIG. 7.—Graphs showing the growth of tadpoles of *Rana aurora draytoni*. The line marked "Normal Control" indicates the growth rate of an unoperated specimen into which no implant was made. The two other lines show the growth rate of hypophysectomized tadpoles into each of which an implantation of an anterior lobe of the hypophysis had been made at the time marked by an arrow. Ordinates indicate total body length in millimeters and abscissae mark the time from the beginning of the experiment measured in weeks.

the volume of its own? The graph shows a slight acceleration of growth, but not a significant one. This indicates that there are certain size limits beyond which growth cannot be pushed by this means. There can be a restoration to normal growth in the cases of tadpoles

retarded by hypophysectomy, but little increase on the part of tadpoles in which the anterior lobe of the hypophysis has been functioning.

Figures 5, 6, and 7 show most interesting and instructive cases in that we see the sluggish growth of hypophysectomized individuals up to the time at which hypophysis implants are made. In those cases where the intermediate or posterior lobe was implanted, no change in the curve of growth results. The case is decidedly dif-

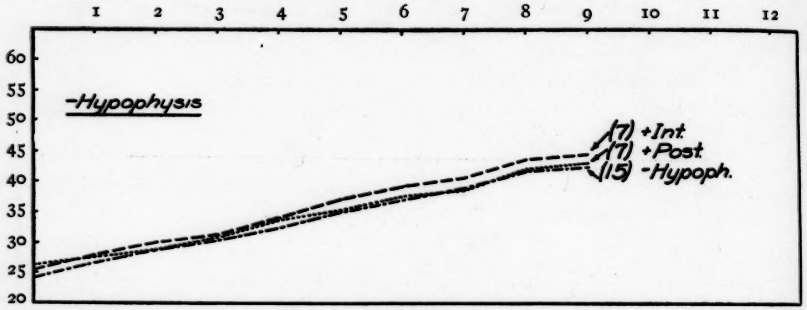


FIG. 8.—Graphs indicating growth of hypophysectomized tadpoles of *Rana aurora draytoni*. The line labeled "+Int." indicates growth of those into which the intermediate lobe of the hypophysis had been implanted. "+Post." shows the growth of tadpoles into which the posterior lobe of the hypophysis had been implanted. Those labeled "—Hypoph." have no implants. Ordinates indicate total body length in millimeters and abscissae mark the time from the beginning of the experiment measured in weeks.

ferent where the anterior lobe of the hypophysis is implanted. In these there is a very marked response which carries the growth curve to normal as shown in Figure 7. It will be seen that the slower growing tadpoles were selected for implantation of the anterior lobe of the hypophysis, thus making their reaction all the more convincing. Although these cases are few in number, they are very significant. Specimens used were selected from the same container, the material implanted in each group was taken from the same animal, and the lots were reared under conditions as nearly identical as possible.

Because of the small number of specimens in certain groups studied in 1925, much of the work was repeated in 1927. Figures 8

and 9 give the results. Little need be added by way of explanation. In Figure 8 we note a very close correspondence between the growth of hypophysectomized tadpoles without implant and that of hypophysectomized tadpoles with implants of the intermediate lobe of the hypophysis and with the posterior lobe. We have here good substantiation of the results attained in the 1925 experiments. The same may be said regarding the implants of the anterior lobe of the hypophysis into thyroidless tadpoles. The same principles are found to apply here as in the earlier series while the numbers are

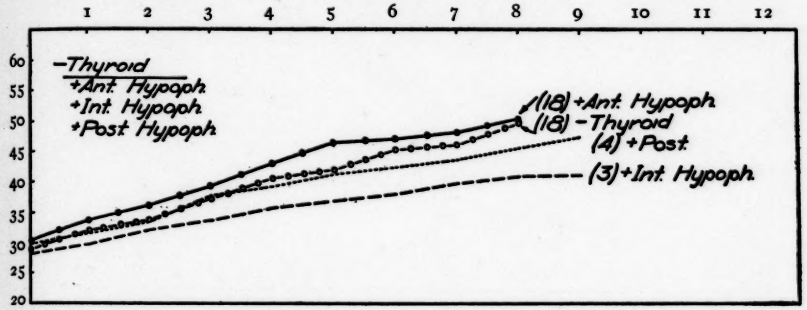


FIG. 9.—Graphs showing the rate of growth of tadpoles of thyroidless *Rana aurora draytoni* studied in 1927. The line marked "—Thyroid" represents the growth of tadpoles from which the thyroid gland alone had been removed, and into which no implantations had been made. Implantations are indicated as in Fig. 4.

sufficient to fully reinforce the previous work. Thyroidless tadpoles into which implants of the intermediate lobe and posterior lobe of the hypophysis had been made were so few as to have little value, the chief discrepancy being that they did not show even as rapid growth as did the thyroidless specimens into which no implant had been made. (Fig. 9).

A very few experiments were made to test the influence of implants of the anterior lobe of the hypophysis into tadpoles from which *both* the hypophysis and the thyroid gland had been removed. The graphs (Fig. 10) give the results of this work. They have little significance because of the small number of specimens involved, but they tend to show that implantation of the anterior lobe of the hypophysis causes some increase in growth over control specimens,

both the implant and control lots showing a higher rate of growth than do the specimens from which the hypophysis and thyroid glands have been removed.

## DISCUSSION OF RESULTS

The failure to produce material increase of growth in these specimens with an already adequate anterior hypophysis by means of an additional anterior lobe implant is one of the most significant results of these experiments because it shows that there are limits to the capacity of an organism to respond to growth stimulus beyond a

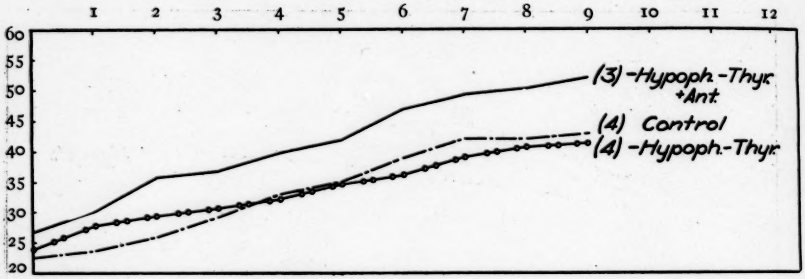


FIG. 10.—Graphs showing the growth of tadpoles of *Rana aurora draytoni*. In all cases the thyroid gland had been removed. The line marked "+Ant. Hypoph." indicates the growth of tadpoles into which the anterior lobe of the hypophysis of an adult frog had been implanted. This added its secretion to that of the anterior lobe of the hypophysis already present. Ordinates indicate total body length in millimeters and abscissae mark the time from the beginning of the experiment measured in weeks.

certain point. Smith (1920) found this true in his feeding experiments. In the later work of Smith and Smith (1923), in which they made intraperitoneal injections of emulsions of the anterior hypophysis of cattle, they produced much more pronounced results, although it is difficult to compare the calculations of volume in this later work with the measurements of the earlier paper. It may be possible that frequently repeated implantations of the anterior lobe of the hypophysis may further increase the growth response; but it is quite certain that the results would not be all comparable to the amount administered.

This work shows that of the materials implanted, the anterior lobe of the hypophysis is alone concerned in inducing size growth

or in restoring it after hypophysectomy, and that the intermediate and posterior lobes are incapable of doing so. No work has thus far been done to determine the relation of the pars tuberalis to growth.

## SUMMARY

1. Growth in length of *Rana aurora draytoni* tadpoles goes on at practically the same rate in normal controls and in thyroidectomized specimens.

2. Hypophysectomized tadpoles lag far behind in growth.

3. Restoration of the anterior lobe is successful in restoring normal growth to hypophysectomized tadpoles, the curve of growth rising rapidly to normal and continuing along the path of growth of normal and thyroidectomized tadpoles.

4. Implantation of the intermediate or posterior lobe of the hypophysis has no effect upon size growth.

5. Implantation of the anterior lobe of the hypophysis into thyroidectomized tadpoles, causes only a slight increase in growth not at all commensurate with the size of the implant.

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## EXPLANATION OF GRAPHS

All represent the rate growth of the tadpoles of *Rana aurora draytoni* under the influence of various combinations of the endocrine glands.

Ordinates represent the total length of the body in millimeters, each interval representing 5 mm.

Abscissae represent the dates of measurement in weeks, beginning with the first length determination made.

−Hypophysis = hypophysectomy.

−Thyroid = thyroidectomy.

−Hypoph.-Thyr. = removal of both hypophysis and thyroid gland.

+Ant. or +Ant. Hypoph. = implantation of the anterior lobe of the hypophysis.

+Int. or +Int. Hypoph. = implantation of the intermediate lobe of the hypophysis.

+Post. or +Post. Hypoph. = implantation of the posterior lobe of the hypophysis.

Numbers in parentheses before designation of implant are used to indicate the number of specimens averaged together in the construction of the curve. Other curves without such numbers indicate the growth of individual tadpoles.

In graphs where −Hypophysis or −Thyroid is written at the left-hand side all the curves in the figure apply to tadpoles from which the gland in question has been removed, and various implants made in cases indicated by a + mark. The control hypophysectomized or thyroidectomized are indicated by −Hypophysis or −Thyroid respectively, placed at the right side of the line indicated.

*X* is placed at the point on a curve that indicates the date on which an implant was made after the beginning of the measurements. In these cases, the nature of the implant in question is indicated by an arrow.

# THE EFFECT OF $CO_2$ ADMINISTRATION UPON PARATHYROID TETANY

(Four figures)

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IT HAS been known for some years that certain mineral acids such as HCl and acid-producing salts such as Ca $Cl_2$ and $NH_4$-Cl are effective agents in the treatment of infantile tetany, and have also been successfully employed in cases of experimental tetany.

Wilson, Stearns, and Thurlow (1915) in a series of experiments performed upon parathyroidectomized dogs observed that injection of 0.1 to 0.5 grams of HCl relieved the tetany symptoms.

György reported (1922) that the administration of such salts as calcium chloride and ammonium chloride gave excellent results in cases of infantile tetany. This has been confirmed by Gamble and Ross (1925) and Anderson and Graham (1924).

Wenner (1927) tested the effect of $NH_4Cl$ on the prevention of experimental tetany in dogs, and the action of this acid-producing salt on the serum calcium level of parathyroidectomized animals. He noted that $NH_4Cl$ is efficacious in preventing the onset of tetany in operated dogs, and when administered orally in large doses to animals presenting severe symptoms and low serum calcium, it alleviates the symptoms and induces a rise in the serum calcium.

Wenner and Muntwyler (1927) observed during the course of their study of alkalosis and tetany several cases of apparently complete recovery from violent tetany convulsions, the improved condition lasting several hours. Study of the blood chemistry of these cases revealed that the pH and alkali reserve of the blood fall far below the normal—pH 7.09, $CO_2$ content 26.8 vol. per cent in one case.

The effect of injecting HCl and $CaCl_2$ or $NH_4Cl$ is the production of an acidosis. For instance Haldane, Hill, and Luck (1923)

clearly demonstrated that a change in the reaction of the blood toward the acid side follows ingestion of large amounts of $CaCl_2$. The Ca is eliminated through the alimentary tract as calcium carbonate, whereas the chloride is almost completely absorbed and replaces the bicarbonate of the blood thus inducing acid intoxication.

It seemed evident from a survey of the literature on the subject,

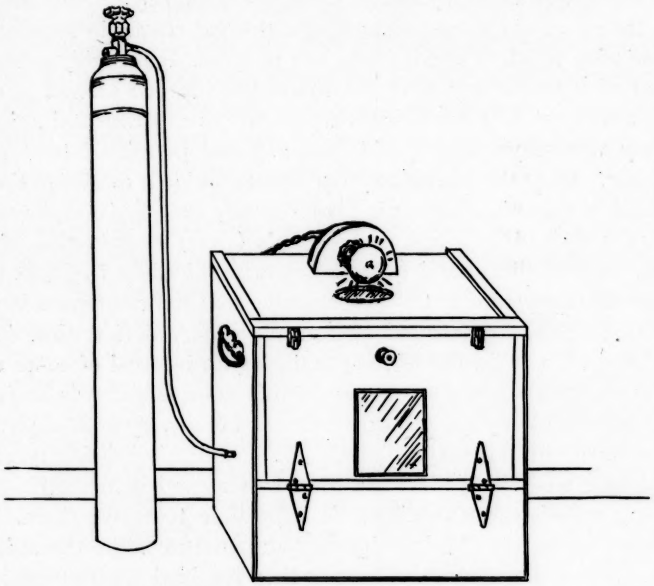

FIG. 1.—$CO_2$ chamber used in the experiments

that the gross manifestations of tetany—even the most violent convulsions—are rather promptly relieved by slight changes in the reaction of the blood toward the acid side, and that such changes influence in some manner the calcium content of the blood. In view of these facts it was considered worth while to test the effect of administering $CO_2$ to dogs in tetany (1) in relation to relief of symptoms, and (2) the effect upon the serum calcium level.

The method of $CO_2$ administration was simple and is illustrated by the schematic drawing of the apparatus employed (Fig. 1). A

specially constructed air-tight chamber was used, the only special feature consisting of a thick glass panel in the front through which the respiration of the animal could be followed, and at the top a small oval glass with an electric light bulb and reflector just above and outside the chamber. No effort was made to determine the exact percentage of gas in the chamber. We were interested chiefly in the effects of asphyxiation upon the tetany syndrome and not in the effect of $CO_2$ per se, and used this gas chiefly because of the ease with which it lent itself to our purpose. It is highly probably that nitrogen or any inert gas would have served as well as $CO_2$.

Small rat and fox terriers were used as experimental animals. They were thyroparathyroidectomized and permitted to develop tetany. When the animals were prostrate and in a condition similar to that of the animal shown in Figure 2, they were bled and the serum Ca determined by Clark and Collip's (1925) modification of the Kramer-Tisdall method (1921). Following bleeding the dogs were immediately placed in the $CO_2$ chamber and sufficient gas administered to produce unconsciousness. We observed that slow intermittent administration of the gas during an interval of 20-40 minutes until unconsciousness supervened is more effective in so far as the relief of tetany symptoms is concerned than very rapid asphyxiation with large quantities of $CO_2$. The animals require constant watching while in the box and must be removed immediately after losing consciousness or else it is impossible to revive them. The second blood was obtained by cardiac puncture while the animals were reviving or very shortly after they regained consciousness.

The rapidity with which the muscle tremors, jerks, and convulsions disappear during asphyxiation is remarkable. Within two minutes following entrance of the gas into the chamber manifest tetany disappears. The struggles of the slowly asphyxiating animal are of a quite different nature from tetany and the difference is obvious to even the casual observer. Our best cases were dogs which developed violent tetany during the day and which had not been prostrate for hours. Our poorest cases in so far as recovery from tetany after asphyxiation was concerned were those dogs in which violent attacks of tetany occurred during the night and the animals were exhausted when placed in the $CO_2$ chamber. Such dogs do not react

in such a striking manner as the cases exhibiting tetany but a short time.

The animals very quickly recover when removed from the $CO_2$ chamber and exhibit no symptoms of tetany. They run about, play, eat, and behave as do normal unoperated dogs. One of our cases engaged in a hearty fight with another dog shortly after removal from the box, whereas but a short time previously he had been prostrate with tetany.

The length of the recovery period varies considerably with the various animals. Some dogs do not again exhibit tetany symptoms for twelve hours or more, others show tremors and even more violent symptoms within a few hours. If such animals are again placed in the $CO_2$ chamber and gas administered, they again temporarily recover. No effort was made to keep the animals alive indefinitely by the experimental procedures employed, since we were primarily interested in the effect of acid intoxication upon the serum calcium and the relief from tetany.

Figure 2 shows a dog prostrate in violent tetany spasms a few moments prior to placing it in the $CO_2$ chamber. Figure 3 shows the same animal one hour after a twenty-minute exposure to $CO_2$. Figure 4 shows the dog twelve hours later. All of the animals used in the experiment died of tetany.

In Table I is shown the effect of the $CO_2$ treatment upon the level of the serum calcium, and the changes in the $CO_2$ content, $CO_2$ capacity, and pH of the blood a few minutes after removal from the chamber. Determinations of the serum calcium made later (1-4 hours) after asphyxiation by $CO_2$ showed no changes in the serum calcium, but a return to approximately normal in so far as the $CO_2$ content, $CO_2$ capacity and pH of the blood were concerned.

It is quite evident from the figures given in Table I that the dogs were suffering from a marked acid intoxication when removed from the $CO_2$ chamber. The changes in the $CO_2$ capacity, content, and pH of the blood clearly indicate acidosis. It is also obvious that the acidosis present was not due to accumulation of $CO_2$, for the $CO_2$ capacity, and content of the blood was invariably low and this holds true for the pH also. The results indicated that some organic acid was probably responsible for the observed blood changes. Since the

FIG. 2.—Dog in violent tetany before $CO_2$ administration
FIG. 3.—Same dog as shown in Figure 2 after $CO_2$ treatment
FIG. 4.—Same animal twelve hours after treatment

TABLE I

| Animal | Tetany Dogs before $CO_2$ | | | | | Tetany Dogs after $CO_2$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Symptoms | Mgm per 100 c.c. Ca | $CO_2$ Capacity | $CO_2$ Content | pH | Symptoms | Mgm per 100 c.c. Ca. | $CO_2$ Capacity | $CO_2$ Content | pH | Remarks |
| 1..... | Violent tetany | 5.9 | ..... | ..... | ..... | None | 6. | ..... | ..... | ..... | $CO_2$ given for 20 min. |
| 2..... | Violent tetany | 6.7 | ..... | ..... | ..... | None | 7.2 | ..... | ..... | ..... | $CO_2$ given for 1½ hrs. |
| 3..... | Violent tetany | 6.4 | ..... | ..... | ..... | None | 7. | ..... | ..... | ..... | $CO_2$ given for 32 min. |
| 4..... | Violent tetany | 6.5 | ..... | ..... | ..... | None | 7.6 | ..... | ..... | ..... | $CO_2$ given for 26 min. |
| 5..... | Violent tetany | 5.9 | ..... | ..... | ..... | None | 6. | ..... | ..... | ..... | $CO_2$ given for 19 min. |
| 6..... | Violent tetany | 6.4 | ..... | ..... | ..... | None | 7. | ..... | ..... | ..... | $CO_2$ given for 45 min. |
| 7..... | Violent tetany | 6.8 | ..... | ..... | ..... | None | 7.1 | ..... | ..... | ..... | $CO_2$ given for 23 min. |
| 8..... | Violent tetany | 5.7 | ..... | ..... | ..... | None | 5.8 | ..... | ..... | ..... | $CO_2$ given for 20 min. |
| 9..... | Violent tetany | 6.3 | 45.6 | 42.1 | 7.38 | None | 6.8 | 32.1 | 28.1 | 7.21 | Unconscious when taken from chamber |
| 10..... | Violent tetany | 6.7 | 47.3 | 40.6 | 7.39 | None | 7. | 28. | 24. | 7.22 | $CO_2$ given for 20 min. |
| 11..... | Violent tetany | 6.4 | 48.2 | 43.1 | 7.4 | None | 7. | 30.1 | 27.2 | 7.25 | $CO_2$ given for 25 min. |
| 12..... | Violent tetany | 6.5 | 56. | 48. | 7.36 | None | 7.6 | 31.1 | 28. | 7.14 | Unconscious when taken from chamber |
| 13..... | Violent tetany | 6.5 | 50.5 | 43.7 | 7.46 | None | 6.7 | 36. | 32.2 | 7.28 | $CO_2$ given for 24 min. |
| 14..... | Violent tetany | 6. | 45.7 | 38.1 | 7.36 | None | 6. | 28.7 | 26.2 | 7.14 | $CO_2$ given for 45 min. |
| 15..... | Violent tetany | 6.5 | 54. | 45. | 7.38 | None | 7. | 33. | 30. | 7.22 | $CO_2$ given for 45 min. |
| 16..... | Violent tetany | 6.2 | 48.7 | 40.8 | 7.44 | None | 6.6 | 30.9 | 28.7 | 7.19 | Unconscious when taken from chamber |

$CO_2$ treatment was really a simple method of asphyxiation, it seemed probable that lactic acid was the responsible agent. The idea seemed valid in view of McGinty and Gesell's (1925) work on the effect of CO administration upon normal dogs. They observed that asphyxiation by CO causes a striking increase in the lactic acid content of the blood. Their figures for the normal lactic acid of dog blood was 23.9 mgm. per 100 c.c. whereas following CO administration the lactic acid rose to 75.1 mgm. per 100 c.c.

TABLE II

| Animal | Time | Lactic Acid Mgm. Per Cent | $CO_2$ Content Vol. Per Cent | $CO_2$ Capacity Vol. Per Cent | pH | Remarks |
|---|---|---|---|---|---|---|
| | October 30 | | | | | |
| Dog No. 1... | 10:00 A.M. | 28.7 | 33.3 | 43.9 | 7.44 | Violent tetany |
| | 10.35 A.M. | 56.5 | 22.2 | 27.6 | 7.25 | $CO_2$ given for 20 minutes. Recovery |
| | 3:40 P.M. | 27.9 | 35. | 42.7 | 7.45 | Tetany |
| | 4:05 P.M. | 88.9 | 29.2 | 32.7 | 7.29 | $CO_2$ given for 20 minutes. Recovery |
| | October 31 | | | | | |
| Dog No. 2... | 10:00 A.M. | 24.1 | 37.6 | 44.7 | 7.46 | Violent tetany |
| | 10:25 A.M. | 108.5 | 28.8 | 32.4 | 7.28 | $CO_2$ administered 20 minutes |
| | 3:50 P.M. | 23.2 | ..... | ..... | ..... | Tetany |
| | 4:15 P.M. | 60. | ..... | ..... | ..... | Dog unconscious after 20 min. Recovery |
| | 5:00 P.M. | 81.7 | 27.7 | 29. | 7.16 | $CO_2$ given 40 min. |

In Table II are shown the lactic acid, $CO_2$ content, $CO_2$ capacity, and pH changes in the blood of tetany dogs before and following $CO_2$ treatment. We employed Riegel's (1927) modification of the Clausen (1922) sulfuric acid method for determining lactic acid.

From the data presented in Table II it seems clear that the changes in $CO_2$ capacity, $CO_2$ content, and pH of the blood are not due to $CO_2$ per se but to the increase in lactic acid induced by anoxemia resulting from the $CO_2$ treatment. The rise in lactic acid following treatment is striking and consistent, and in our opinion is sufficient to account for the observed changes in pH. The decreased $CO_2$ content and capacity are obviously due to hyperventilation

caused by the $CO_2$ treatment, with consequent blowing off of carbon dioxide.

It will be noted that four lactic acid determinations were made on dog No. 1, and five on dog No. 2. The lactic acid content of the blood is high during the recovery period and the dog remains free from tetany symptoms. When, however, the tetany again becomes manifest lactic acid was found to be at a normal concentration, i.e., about 28 mgm. per cent. The drop in lactic acid might have occurred some time before the first tetany symptoms became manifest.

Macleod (1921) observed that within an hour after asphyxia and anoxemia were terminated the blood lactic acid returned to the normal level. Table II shows that tetany reappeared five hours after the two animals were taken from the $CO_2$ chamber and allowed to breathe outside air. However, some of our cases developed tetany within an hour following treatment. It is very likely that in such cases the lactic acid content of the blood had returned to the normal level very quickly after removal from the $CO_2$ chamber.

Our data indicate that changing the reaction of the blood to the acid side produces an effect upon tetany which persists for some hours following a return to normal of the acid-base equilibrium. The work of Reigel (1927) on the rate of removal of lactic acid following injections of sodium lactate tends to bring out this point. Two hours after injecting an equivalent of 2,125 mgm. lactic acid, the lactic acid of the blood returned to a normal concentration while the inorganic phosphate of the blood remained at a much reduced figure for some time following.

The question arises, how does a shift in the reaction of the blood toward the acid side bring about a cessation of tetany symptoms? It appears probable that such a shift in the acid base balance produces profound changes in certain constituents of the body fluids. Thus Freudenberg and György (1921) have shown that calcium carbonate in solution is able to supply the correct amount of ionic calcium of the blood, the ionic calcium being one fourth the amount of total calcium. Furthermore they have demonstrated that the amount of ionic calcium present in the blood is dependent on the hydrogen ion and bicarbonate concentration. With a decrease in pH there occurs an increase in ionic calcium. Following parathy-

roidectomy it is probable, as Greenwald (1926) suggested, that the fall in total and ionized calcium is the result of a precipitation in the tissue of tertiary calcium phosphate which Holt, La Mer, and Chown (1925) have shown to be present in excess in the blood of normal animals. Stewart and Percival (1927) working on eviscerated cats found a marked rise in total calcium of serum after injection of parathyroid hormone showing clearly that the calcium must come from the tissues and not as a result of an increase in calcium absorption in the intestine.

The increase in lactic acid content of the blood brought about by anoxemia in presence of an excess of $CO_2$ is sufficient to account for the low pH and low $CO_2$ content of the blood. This increased acidity probably increases the ionic calcium concentration and increases the excretion of phosphates. An increased excretion of phosphates after the administration of hydrochloric acid and hydrochloric acid-producing substances to children with tetany has been reported by Freudenberg and György (1921) and Gamble and Ross (1925). Anderson and Graham (1924) found that the inorganic phosphate of the blood is decreased following the ingestion of ammonium chloride.

In the experiments reported here, it is probable that the acidosis caused by asphyxia and anoxemia has a greater effect on the ionized calcium than upon the total calcium. The slight increase in total calcium observed in some cases is probably due to the increased solubility of calcium phosphate at the low pH. The relief of experimental tetany by acidosis is a simple and effective method of treatment, and produces its effect by rendering the available calcium of the blood more diffusible and probably stimulates excretion of excess phosphates.

## PROTOCOLS

Dog No. 38. Rat terrier, female, weight 8.5 kilos. Thyroparathyroidectomized March 22, 1927, 4:00 P.M. Tetany appeared 9:00 P.M., March 25. Animal very spastic, marked tremors and jerking of flank and leg muscles. Panting, salivation. Bled from heart. 15 c.c. taken. Serum Ca 6.5 mgm. per 100 c.c., $CO_2$ content 48, $CO_2$ capacity 56, pH 7.36. Animal placed in $CO_2$ chamber 9:30 P.M. and left there 25 minutes. Removed from chamber unconscious. Bled. $CO_2$ content 31.1 vols. per cent, $CO_2$ capacity 31.4 vols. per cent, pH 7.14. Serum Ca 7.6 mgm. Animal quickly regained consciousness and exhibited no signs of tetany. Ran about the laboratory, ate meat, and engaged in a fight

with another dog. At 11:40 P.M. dog bled again. Serum Ca 7.6. mgm. This animal remained free from any signs of tetany for fifteen hours after removal from $CO_2$ chamber. This dog died of tetany March 28 at 9:00 A.M.

Dog No. 39. Fox terrier, male, weight 7 kilos. Thyroparathyroidectomized March 20 at 2:00 P.M. Remained normal until March 23 at 8:00 A.M. Exhibited violent tetany. Serum Ca 6.7 mgm., $CO_2$ capacity 47.3 vols. per cent, $CO_2$ content 40.6 vols., per cent, pH 7.35. Placed in $CO_2$ chamber for 30 minutes. Taken out unconscious. Bled. Serum Ca 7.2 mgm., $CO_2$ capacity 28 vols. per cent, $CO_2$ content 25.1 vols. per cent, pH 7.23. Animal recovered consciousness within two minutes after removal from chamber. No visible signs of tetany. Animal behaved in normal fashion and remained free from tetany for 7 hours. When violent tetany symptoms again developed the animal was again placed in the $CO_2$ chamber and after removal all tetany symptoms had disappeared. This animal died of tetany thirty-six hours later.

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# THE PHYSIOLOGICAL GRADIENTS IN HYDRA

## I. RECONSTITUTION AND BUDDING IN RELATION TO LENGTH OF PIECE AND BODY LEVEL IN PELMATOHYDRA OLIGACTIS

(Eighty-three figures and four graphs)

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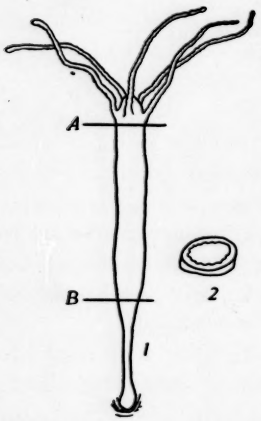
### INTRODUCTION

THE present paper is the first step in an attempted physiological analysis of hydra as an individual with particular reference to the significance of the polar gradient and includes data on reconstitution in relation to length and size of piece and level of the body, on rate of tentacle formation in relation to level, on susceptibility gradients in pieces, and on conditions determining formation and separation of buds. The presentation of data already in hand is reserved for subsequent papers.

A review of the literature on hydra, particularly with regard to previous work on regeneration and reconstitution, seemed to indicate that a further study of this form from a quantitative standpoint and with the theory of the axial gradient in mind, might serve to give new meaning to results previously obtained as well as open up new lines of attack. Since Trembley's classic monograph of 1744 there has accumulated a widely varied and extensive literature relating to various fields of experimentation in hydra. These papers have been carefully summarized and reviewed by Koelitz (1910) and Hyman (1927), and deal with lines of experimentation which are rather remotely related to those to be reported in this paper.

It is well known that in some of the simpler animals the organism resulting from a piece following reconstitution often differs from the parent-animal in regard to morphological pattern. In *Tubularia* and *Corymorpha* forms have resulted ranging from unipolar (uniapical), bipolar (biaxial, heteromorphosis) to multipolar (multiapical) (Driesch, 1899; Morgan, 1902, 1903; Child, 1926, etc.). Similar re-

sults have been reported for *Planaria* by various investigators. An analysis of this work by Child (1915, 1924) indicates that these various forms are manifestations of definite dynamic states which control the polarity and symmetry of the resulting organism through the persistence of pre-existing, or the determination of new gradients and relations of dominance and subordination in various regions. Some preliminary incompleted work by A. E. Galigher, a student in this laboratory, has served to point out the possibility of throwing some further light on the problem of metabolic gradients in hydra by the study of the differential reconstitution of pieces of various sizes from different levels of the body.




FIGS. 1–2.—Large nonbudding *P. oligactis* and a typical body section.

The writer desires to express his sincere thanks and appreciation to Professor C. M. Child, who suggested this problem, and to both him and Dr. L. H. Hyman for many helpful suggestions and kindly criticisms in carrying out the experiments.

## METHODS AND MATERIAL

The animals used in these experiments belong to the species recently classified by Schulze (1917) as *Pelmatohydra oligactis* (Schulze) Pallas (= *Hydra fusca* Linnaeus, *Hydra oligactis* Pallas, and *Hydra dioecia* Downing). Schulze has made a careful analysis of the morphological features of the various species of hydra, and has based his reclassification upon differences in color, the presence or absence of a well-defined stalk, and, lastly, the differences in the kinds of nematocysts found in the various species. This species of hydra is the large brown form which has rather long tentacles, a distinct brown body region much larger in diameter than the slender almost colorless stalk at whose end is the round foot by which the animal is attached (Fig. 1). This animal agrees in practically every detail with the description given above by Schulze for *Pelmatohydra oligactis* and has been thus identified by Dr. L. H. Hyman.

The stocks used in these experiments were raised in the laboratory from animals collected in June, 1926, from a lagoon in Jackson Park, Chicago, Illinois. They were brought into the laboratory with some *Elodea* and *Ceratophyllum* and from a stock of some twenty animals only seven survived the period of depression which ensued when they were first brought under laboratory conditions. From these seven animals the stock cultures of hydra used in the following experiments were derived by budding.

The animals were kept in large battery jars and glass aquaria containing aerated well water and some vegetation—Algae and a small amount of *Elodea*. They were abundantly fed daily with *Daphnia* which were raised in the laboratory in cultures made up from boiled pond weeds in well water. To these cultures at varying intervals were added some small pieces of liver. Care was taken not to foul the water by adding too much liver. The procedure followed for cultivating hydra and *Daphnia* is given in detail by Hyman (1927). During the summer months the stock jars of hydra were kept on a water table surrounded by water and by this means the temperature of the culture water was kept at or below 25° C. In the winter the animals flourished very well at room temperature.

Sectioning of the animals was carried out as follows. They were removed a few at a time from the culture by means of a dropper and transferred into a stender dish containing water from the culture. From this they were removed to the concave bottom of an inverted finger bowl which, when filled, contained sufficient water to allow normal expansion of the animal. The hydra was allowed to attach, and after it had expanded as fully as possible the hypostome and tentacles were first removed and sections of the body of desired length or size were cut from the apical region (Fig. 1*A*) toward the basal limit of the body (Fig. 1*B*). Since the stalk would not regenerate it was discarded. The piece of the body region thus removed by section was a complete circle of body tissue as shown by Figure 2. Since violent contraction followed each section, when dividing the animal into a number of pieces of as nearly equal size as possible, it was necessary to wait until the specimen expanded again before making another section. The instrument found best adapted for sectioning in these experiments was a small scalpel with

a somewhat triangular cutting edge which is fully described as No. 3996 in the *Catalogue* of the Central Scientific Company.

Various surfaces other than glass, such as paraffin and wood, were tried for cutting, but these, besides being objectionable from the standpoint of contamination both chemically and bacteriologically, were so soft that the blade of the scalpel sank into them very readily and with it a portion of the section which was dislodged only with difficulty and in many cases with injury. With the glass substrate sterilization was not difficult, and the hard surface permitted clean-cut sections to be made which would float away from the parent-piece. These advantages more than compensated for the time spent in sharpening scalpels.

After cutting, the sections were transferred by means of a dropper to small stender dishes, size 25×5 cm., which had a capacity of 32 cc. These dishes had been previously sterilized by boiling for fifteen minutes in distilled water, after which they were placed in a closed glass vessel. The sterilized dishes were filled about one-third full of "prepared" well water or, in later experiments, with "prepared" culture water. The water from the well in the basement of the Hull Zoölogical Laboratory, which has been found by chemical analysis to contain 49.2 mg. of Ca per million besides various other elements, is prepared by boiling vigorously for fifteen to twenty minutes in pyrex flasks. It is then allowed to stand for about twelve hours, after which the upper two-thirds is carefully siphoned off. A chemical analysis of the boiled well water shows that it differs from ordinary water only in the amount of Ca present which has been reduced from 49.2 parts per million to 26.1.[1] The boiled decanted water is then aerated by shaking before being poured into the stender dishes. This water can be allowed to stand for any length of time without deleterious effects on the cut pieces. "Prepared" culture water is water which has been removed from the stock animal cultures and filtered through three layers of soft filter paper. This serves to remove decaying organic matter and most of the Protozoa which are always found in abundance in the hydra cultures. The filtered water is then aerated by shaking.

[1] The Department is indebted to A. M. Buswell, of the Illinois State Water Survey Division, Urbana, Illinois, and to Mariner and Hoskins, Chemists, Chicago, Illinois, for making these analyses.

The experimental work was begun by using the prepared well water, but later in the year it was found that the pieces either failed to reconstitute or, at best, underwent depression within a few days in this water. This may have been due to some change in the chemical content of the well water, for during the summer months there was little of this water used in the building, but in the fall and winter the water was used in large amounts and at one time there was difficulty in obtaining sufficient water from the well. Moreover, it has been observed that entire animals in the early stages of depression would recover within a few hours if placed in another culture in which the animals were in good physiological condition. The same was found to hold true for pieces in depression, i.e., a transfer from well water to culture water would arrest depression. It was then decided to use prepared culture water, and in this the pieces lived as well, if not better, than in the prepared well water. This apparently indicates that the most important advantage derived from boiling the water is not due so much to decrease in bacterial flora as to change in salt content, though the former, no doubt, is an important factor in preventing reconstitution. This assumption is further substantiated by the results of some preliminary work on the reconstitution of pieces in water containing various combinations of different salts. Here there seem to be very good indications that certain salts in excess and in various combinations with other salts have definite effects on the organism. Thus the presence of excess amounts of certain salts in the culture water may at first be the cause of depression but after a time recovery may result, perhaps because of the neutralization of the toxic action of the salts through the excretion of some substance or substances by the animals into the water or because of acclimation to the new environment on the part of the organism, or both. Evidence in favor of the first assumption may be seen in the fact that animals or pieces in good physiological condition when transferred to fluid from another culture in good condition undergo no depression or, if in the earlier stages of depression, recover rapidly. Inasmuch as the water in the stock laboratory cultures has not been previously boiled and thus has a high salt content as analysis shows, it would seem that some alteration in environmental conditions relative to salt content has taken place because of the presence of the animals. Dr. W. C. Allee permits

me to state that unpublished observations by him on *Ophioderma brevispina* and on certain fishes seem to indicate something similar under somewhat the same conditions.

The stender dishes containing the cut pieces were placed in low earthenware dishes whose bottoms were covered with a small amount of water. These were placed in water on the water table and in this way it was possible to keep the pieces undergoing reconstitution at a fairly constant temperature. The temperature for the most part was about 20° C. and the maximum fluctuation was from 18° to 23° C. Daily observations were made in almost all experiments.

### CLASSIFICATION OF FORMS RESULTING FROM RECONSTITUTION

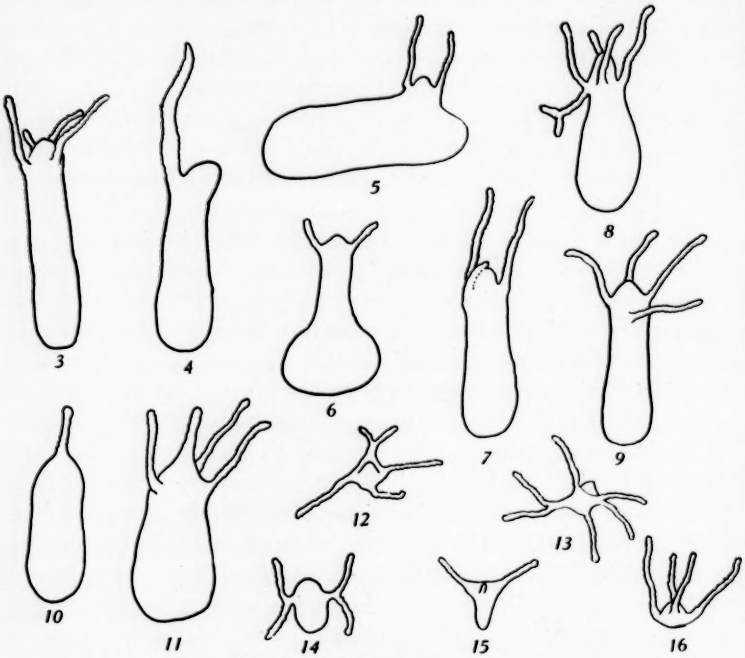
The reconstitution of the pieces of the body region of *P. oligactis* results in a variety of forms differing from one another with respect both to the structures present and their axial relations. These forms are so similar to those obtained by Child (1926*b*) in *Corymorpha* that it has been possible to use, with some modifications, the same general classification for reconstituted forms as that used by Child. In general, the pieces following reconstitution seem to fall into three main classes or groups: unipolar, bipolar, and multipolar forms. Child has broken up the multipolar forms into a number of smaller groups, since in *Corymorpha* duplications of the basal as well as the apical levels occur. In consequence of the relatively larger size of the pieces of *Corymorpha* it was usually possible to establish with certainty the position of the reconstituting apical and basal levels with respect to the cut ends. The small size of the pieces in hydra and the early closure of the cut ends made it often impossible to establish beyond doubt the place of development of the new axes. It seemed more desirable, therefore, to classify all forms having three or more polar axes as "multipolar" forms.

The figures are drawn from living individuals resulting from reconstituted body pieces and show, as accurately as possible, the number and arrangement of tentacles as well as the axial arrangement of parts. In most of the figures it will be noted that there is no stalk and foot present. Since in most cases the pieces were not kept under observation for a period longer than eight to ten days, there was not sufficient time for the formation of these structures,

which, according to Grosz (1925), requires a month in *P. oligactis*. Burt (1925) states that a foot forms on a stalk attached to two-thirds of the body region after eight days. My own observations show some isolated cases of stalk-and-foot formation, the frequency depending upon the size of the piece and the level of the body, pieces from the more basal levels forming them more frequently than those from the more apical. The average time observed was from ten to fourteen days. Presumably, if the pieces had been kept under observation for a longer period of time under favorable conditions stalk and foot would have formed. Consequently, a reconstituted form lacking only stalk and foot will not be regarded as a "partial form" as the term is generally understood, but as a complete individual.

*Unipolar forms.*—Those reconstituted pieces having only one more or less perfect hydranth and body, i.e., one main axis of polarity, are classified as unipolar forms. The term "hydranth" is understood to refer to the hypostome and the surrounding peristomial region with the tentacles. These forms may be (1) complete individuals, i. e.,with hypostome, body, stalk and foot; (2) those lacking a stalk and foot; and (3) unipolar partial forms. The last named consist of only hypostome and tentacles with or without a very small portion of the body region. They result from the smallest pieces from the most apical regions. The two former categories arise for the most part from the more basal levels in the smaller pieces (see the following tables) and from the larger pieces comprising from one-half to the entire body region after removal of hypostome and stalk. Typical examples of unipolar forms are seen in Figures 3–16. Figure 3 shows a complete individual; Figure 4, an individual with a hypostome and one abnormally large tentacle. Figures 5 and 6 show forms in which the hypostome and tentacles arise at an angle to the body but represent only one main axis. These forms are found for the most part in the more basal levels. Figure 7 shows a case of incomplete separation of tentacle and hypostome; Figure 8, branched tentacles; Figure 9, a case of slight irregularity of tentacle position. Figures 10–11 are instances where a tentacle replaces a hypostome. The forms just described are complete or almost complete individuals and occur for the most part at

the more proximal levels of the body in the shorter pieces, and at any level in the longer pieces. The forms shown in Figures 12–16 are from the most distal body regions and occur when the body is cut into tenths or smaller pieces in a manner to be described later. These may very properly be regarded as unipolar partial forms.

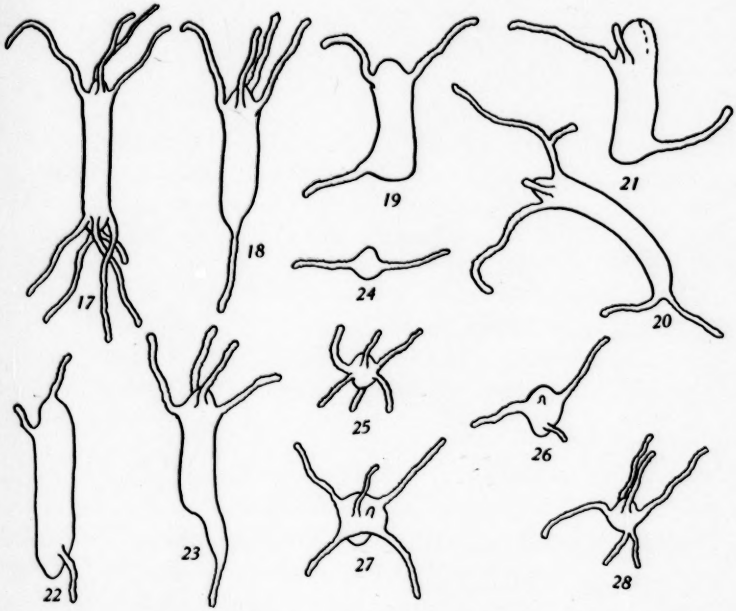



FIGS. 3–16.—Unipolar forms

Figure 12 shows a hypostome surrounded by four tentacles, one of which is branched; Figure 13, a hypostome and four tentacles, two of which are branched; Figure 14, a hypostome with two branched tentacles and a very small body region; Figure 15, a reconstituted piece with the hypostome missing and only three tentacles present; Figure 16, a piece consisting of a hypostome and four tentacles only.

*Bipolar forms.*—Included in this group are those forms having only one complete or incomplete hydranth at each end, presumably

developed at the proximal and distal cut surfaces of the piece with no indication of a lateral developing basal region. The various types of bipolar forms are shown by Figures 17–28. Figure 17 shows a reconstituted piece with a complete functional hydranth on each end. After reconstitution this form was fed at different times. The



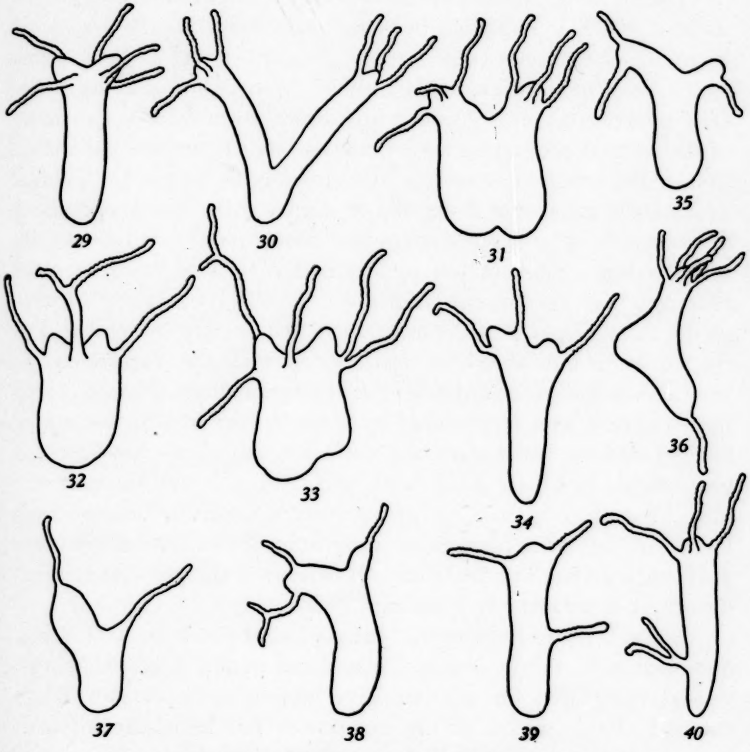

FIGS. 17–28.—Bipolar forms

pieces of a *Daphnia* or a *Cyclops* were grasped by the tentacles, conveyed to the hypostome, and engulfed. Each end was fed at various times. Figure 18 shows a piece with a perfect hydranth with four tentacles on one end and on the other one tentacle only with no indication of a hypostome; Figure 19, a piece with a perfect hydranth at one cut surface and one tentacle arising lateral to the other rounded end; Figure 20, a form similar to bipolar forms previously described except that there is one branched tentacle in the circlet surrounding the hypostome. Figures 21–22 show various

degrees of fusion of hypostome and tentacles at one end and one tentacle in varying positions at the other. Figure 23 shows a piece with a complete perfect hydranth at one cut surface and a lateral elongation from the other ending in a tentacle. Bipolar partial forms are seen in Figures 24–28. Attention may be called to the almost complete absence of any body region between the more or less perfectly developed hydranths. Figure 24 shows two hypostomes with two tentacles in common, developed equidistant between them; Figure 25, two hypostomes each with its circlet of three tentacles; Figure 26, one hypostome with three tentacles of varying size and one hypostome with one tentacle. Figures 27–28 show reconstituted pieces with a perfect hydranth at one end and one or more tentacles only at the other. Examination of the literature on hydra has not shown thus far that bipolar forms have been previously observed.

*Multipolar forms.*—Those forms showing three or more polar axes, at least two of which are indicated by a more or less perfect hydranth or tentacles, have been classified as "multipolar" forms. These forms occur in the highest percentages in pieces consisting of one-half to one-tenth of the body region, and are found in greatest frequency in reconstituted pieces from the distal two-thirds of the body. This group may be subdivided, according to the probable manner of origin and the general type of the reconstituted piece, into two subgroups, the bipolar-unipolar and the multiapical forms. The bipolar-unipolar forms (Figs. 29–40) are those which have two complete or incomplete hydranths with a single proximal or basal level common to them. It has not been definitely determined whether these forms are the result of development of two apical ends at one cut surface with the differentiation of a basal region at the other or whether they have arisen from the reconstitution of a complete or partial hydranth at each cut surface with the formation of a lateral basal region. The latter type of reconstitution has been described by Torrey (1910) and Child (1926) in *Corymorpha*. The morphological patterns of different pieces seem to indicate that either mode of origin is possible. Figure 29 shows a piece which might have originated in the manner first mentioned, and Figure 30, a piece which might be the result of the second method. Figures 29–31 show reconstituted pieces with two perfect hydranths and

one well-defined base, though in the latter case there are some indications of doubling which soon disappeared. Figures 32–33 show the development of hydranths in such close proximity to each other that



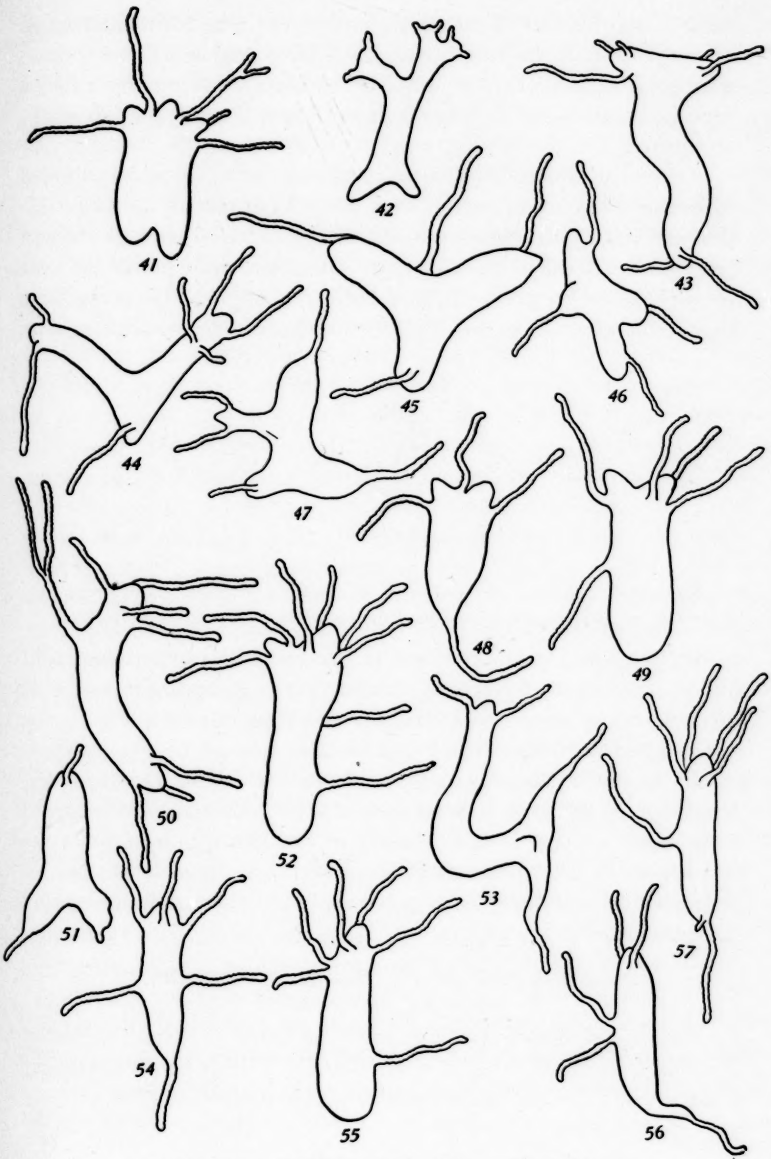

FIGS. 29–40.—Multipolar forms of the bipolar-unipolar type

an abnormally large tentacle results whose double nature is indicated in one case by its bifurcation (Fig. 32). Figures 34–35 show specimens with two perfect hydranths whose tentacle number is variable. The hydranths are at different angles to each other and at various distances from the base. Some bipolar-unipolar forms may have but one complete perfect hydranth, the other consisting of a hypostome-like structure with a tentacle on its tip (Figs. 36–38). Other types

found have one apical region represented by a perfect hydranth, but the other is reducd to one or two tentacular outgrowths (Figs. 39–40).

The multiapical forms are those showing three or more polar axes each of which is indicated by a more or less perfectly developed hydranth or tentacles (Figs. 41–57). Figures 41 and 42 show forms which may have originated by the differentiation of three hypostomes on one cut end. Again, a multiapical form may be the result of the formation of a single hydranth on one cut surface and a division of the other to form two hydranths as in Figure 43. Figures 44–47 show forms which may have originated by the development of hydranths at each cut surface and one or more new lateral axes each having a more or less perfect hydranth. One or more extra tentacles may be present indicative of other polarities. Figures 48–51 show reconstituted pieces with two perfect hydranths and the third axis indicated by tentacle formation only. Figures 44–46, 51–52, show indications of having four apical regions. Figures 53–57 have but one axis represented by a perfect hydranth, the others being indicated by one or two tentacles only. The reconstituted piece shown in Figure 53 is worth noting since it was the only one of its kind found. It consists of a perfect hydranth at one end with the other end of the piece bifurcating to form two stout body processes, each ending in a tentacle. Just back of the point of bifurcation there is formed a hypostome.

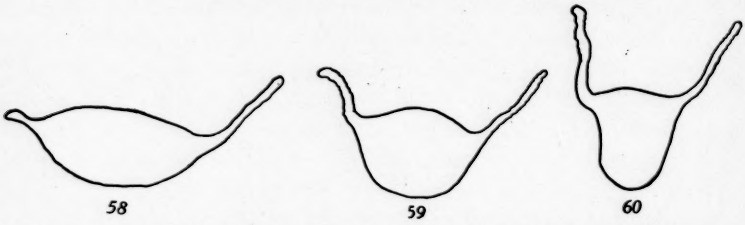
As has already been noted, these forms have been kept under observation in only a few instances for a period longer than two weeks. Very often the animals have undergone depression before the end of this period or the experiment has been discontinued. Because of the small size of the reconstituting pieces, attempts to feed them were made only in a few instances, since it would have been necessary to do this by hand, a very tedious and time-consuming undertaking (see Hyman, 1927). Consequently, the reconstituted pieces are in a starved condition, and it may be for this reason that in all cases of regulation observed following reconstitution, there has been a tendency to approach the unipolar normal condition by a resorption and fusing of the accessory structures indicative of two or more axes. Accessory tentacles are gradually re-




FIGS. 41–57.—Multipolar forms of the multiapical type

sorbed, and several instances were observed where two hydranths of a bipolar-unipolar form gradually approached and fused to form a unipolar animal. Double tentacles regulate to normal by a fusion process much more rapidly than do other double and accessory structures.

One very interesting case of regulation occurred which differed from the foregoing in that it took place by means of a reorganization of existing structures rather than by fusion. Figure 58 shows a piece with a tentacle on each end. These two ends gradually bent toward each other (Fig. 59) until both were apparently on the same end of the piece (Fig. 60). Unfortunately, no other sketches were




FIGS. 58–60.—A case of reconstitution in which a tentacle is formed presumably at the two cut ends and the hypostome from the lateral region of a body section.

made, but I find in the protocol of this particular experiment that all the reconstituted pieces of this particular group are recorded as being "normal" for the remainder of the observations following the time when the piece was last sketched.

In the case of pieces undergoing depression followed by recovery, regulation of the form toward normal after reconstitution occurred much more rapidly in consequence of the absorption of tentacles which always takes place in this condition. These observations on depressed hydras have been made by a number of investigators since the time of Trembley.

### FORM FREQUENCIES IN RELATION TO LENGTH OF PIECE AND BODY LEVEL

The data summarized in the following tables have been collected at different times in the past year. Usually a single series consisting of six or eight hydras was selected from the culture and the animals

sectioned in the way desired. This procedure would be repeated some days later, perhaps, from another culture, consequently the tables include data on animals from different cultures at different times during some nine months. Some variations in frequencies of the different forms appear in the different series, but there is no evidence of progressive change in any direction in the stocks.

In choosing animals for the various experiments attempts were made to select those of uniform age and consequently physiological condition as well as size. After working with *Pelmatohydra* for a short time one is able to recognize very readily a young animal, i.e., one which has recently separated from the parent by budding. In the young specimens the stalk is not so sharply marked off from the body in contour and the pigmentation is still very noticeable in the stalk. In an older animal the stalk tends to lose its color, becoming more white and opaque while the body becomes darker brown in color and much larger in diameter. As the animal ages the stalk tends to become relatively longer than the body. The younger animal has a much shorter stalk in proportion to the body, and because of its greater plasticity may appear as long as, if not longer than, an older individual. Hence if the animals were selected on the basis of size, both the body length and the physiological condition would vary.

The following data refer to the reconstitution of pieces of various size and length from different levels of the body only—from the region under the tentacles to below the budding zone (Fig. 1*A–B*). The writer finds as did Goetsch (1920), Grosz (1925), and Issajew (1926) that the stalk region alone will not regenerate a hypostome and tentacles. In the course of the present experiments some instances of tentacle and hypostome formation on pieces of the stalk have been observed, but since the pieces were from the more distal levels it may be that some body tissue remained on the piece. There is no doubt that a piece of the stalk will reconstitute a hydranth provided there is some body tissue available. Burt (1925) was able to get a hydranth to form on the more proximal regions of the stalk by grafting a small piece of the body tissue to it. Pieces of the stalk from the lower levels regenerate a foot at each end but never more

apical regions. Hence he concludes that the stalk is a unipotent system.

Table I gives the form frequencies in percentages of unipolar, bipolar, and multipolar forms with respect to length of piece and body level for the largest hydras which show no external evidence

TABLE I

COMPARISON OF PERCENTAGE FREQUENCIES OF THE VARIOUS FORMS WITH RESPECT TO LENGTH OF PIECE AND BODY LEVEL IN NON-BUDDING ANIMALS

| Body Length Divided In | Body Level | No. of Pieces | Unipolar | Bipolar | Multipolar | Budding | No Reconstitution or Death |
|---|---|---|---|---|---|---|---|
| Entire | ..... | 33 | 100 | ..... | ..... | ..... | ..... |
| Halves | 1 | 33 | 73 | ..... | 24 | 3 | ..... |
| | 2 | 17 | 53 | ..... | 12 | 35 | ..... |
| Thirds | 1 | 45 | 51 | 13 | 36 | ..... | ..... |
| | 2 | 45 | 51 | 7 | 11 | ..... | 51 |
| | 3 | 45 | 54 | ..... | 2 | 44 | ..... |
| Fourths | 1 | 66 | 33 | 26 | 41 | ..... | ..... |
| | 2 | 66 | 55 | 14 | 28 | ..... | 3 |
| | 3 | 66 | 70 | 7 | 21 | 1 | 1 |
| | 4 | 66 | 83 | 1.5 | 5 | 9 | 1.5 |
| Sixths | 1 | 44 | 18 | 29 | 53 | ..... | ..... |
| | 2 | 44 | 25 | 34 | 41 | ..... | ..... |
| | 3 | 44 | 50 | 23 | 27 | ..... | ..... |
| | 4 | 44 | 53 | 27 | 20 | ..... | ..... |
| | 5 | 42 | 70 | 12 | 16 | 2 | ..... |
| | 6 | 44 | 79 | 2 | 10 | 8 | 1 |
| Tenths | 1 | 41 | 39 | 39 | 19 | ..... | 3 |
| | 2 | 40 | 40 | 47 | 10 | ..... | 3 |
| | 3 | 40 | 35 | 42 | 23 | ..... | ..... |
| | 4 | 39 | 44 | 15 | 36 | ..... | 5 |
| | 5 | 39 | 49 | 26 | 22 | 3 | ..... |
| | 6 | 28 | 68 | 18 | 14 | ..... | ..... |
| | 7 | 26 | 74 | 12 | 14 | ..... | ..... |
| | 8 | 26 | 85 | 4 | 11 | ..... | ..... |
| | 9 | 31 | 93 | 5 | ..... | ..... | 2 |
| | 10 | 22 | 95 | 5 | ..... | ..... | ..... |

of budding, though such animals are no doubt about ready to bud. The first column of the table gives the fractions of body length (Fig. 1*A–B*) which the pieces represent; column 2, the various body levels numbered in order from the distal to the proximal end; column 3, the total number of pieces for each group. Columns 4, 5, and 6 show the frequencies of the various forms in percentages. In column 7 are recorded in percentages the numbers of pieces at various levels which formed a well-defined bud. A piece was classified as budding when the outgrowth with its hydranth began to show evidence of

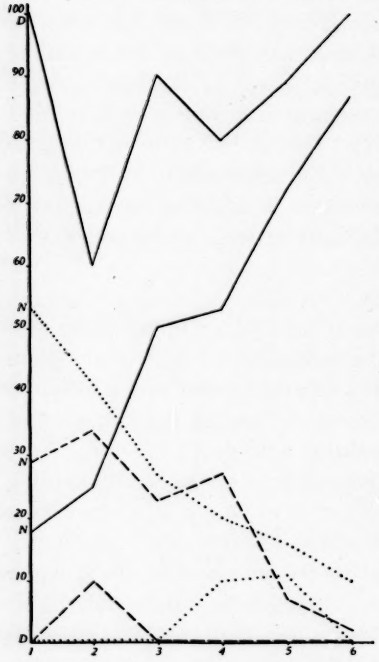
stalk differentiation. This could be seen after two or three days. Complete separation of the buds took place within three to five days after their appearance. The budding piece, if it reconstituted either before or following the separation of the bud or buds as the case may be, always gave rise to a unipolar form. However, the percentage of the reconstituted budding pieces was never added to that of the unipolar pieces for that particular level in the tables. Some pieces gave rise to as many as three buds and were so reduced that they failed to reconstitute. The last column shows in percentages the number of pieces which either fail to reconstitute or die during the course of the experiment. Attention is called to the fact that the percentages of deaths and failures to reconstitute taken together are very low in most cases.

A study of Table I shows, first, that pieces consisting of the entire body region after the removal of the hydranth and stalk form 100 per cent unipolar forms. With decreases in length of the piece to one-half and one-third the body length, bipolar and multipolar forms appear, and with further decrease in length the frequency of these forms increases in the more distal regions.

The data in Table I for form frequencies of sixths of non-budding hydras in good condition are represented in Graph 1 by the curves lettered *N*. The unbroken line *N* shows the increase in frequency of unipolar forms from the distal to the proximal levels, and the broken line *N* and the dotted line *N* show for the bipolar and multipolar forms, respectively, a relatively high frequency in the distal levels and a decrease in the proximal direction. The sixths have been selected for graphing, first, because they are the shortest pieces available for comparison under the different experimental conditions; second, because they give rise to all the different forms; and, third, because they show the widest range in difference of frequency with difference in level and under different physiological conditions. However, as Table V will show, there is no definite relation between total frequency of any form and length of piece. Except in the halves, the frequency of the unipolar forms is, in general, lowest at the distal levels and increases proximally in pieces of a given length, but there is no evident relation between unipolar frequency and length of the piece within the limits of the tables.

In the table, pieces which gave rise to buds, i.e., to new axes

which separated from the parent-piece, are listed under the column headed "Budding." As the table shows, such pieces are from the proximal body levels, the region of the budding zone. Pieces which give rise to buds sometimes fail to undergo reconstitution or their reconstitution is much delayed, but the majority do reconstitute sooner or later. When they do they give rise to unipolar forms. If such cases listed in Table I under "Budding" were included among the unipolar forms, the frequencies of these forms in the proximal regions would be further increased. In Graph 1 the unbroken line *N* for the unipolar forms represents the combined frequency percentages of the unipolar and the budding pieces. Budding, however, occurs much less frequently in the sixths and shorter pieces than in the halves or thirds.




GRAPH 1.—Graph showing the form frequencies at six body levels of non-budding *P. oligactis* in good condition and in depression from the data in Tables I and III. The ordinates represent percentage form frequencies and the abscissae the body levels of the animal, 1 representing the most apical. The curves lettered *N* are drawn from pieces in good condition and those lettered *D* from depressed pieces. The solid lines ——— represent the frequencies of unipolar forms; the broken lines – – – –, bipolar forms; and the dotted lines ....., multipolar forms.

Table II summarizes the data on form frequency of pieces of various lengths from different body levels of large budding animals. Hydras of uniform size were selected and the buds removed as close to the parent-animal as possible without injury to it, after which the body was sectioned as desired. The budding animal differs from the non-budding in that it is somewhat older and the body, though

of approximately the same length, is larger in diameter. A comparison of the same levels in Tables I and II shows that there are no marked frequency differences. Unipolar frequency increases and bipolar and multipolar frequencies in general decrease in the proximal direction.

### EFFECT OF DEPRESSION ON FORM FREQUENCY

The condition known as "depression" in hydra has been observed and described frequently by European zoölogists. The best of these accounts are those of R. Hertwig (1906) and Frischholtz (1909).

TABLE II

COMPARISON OF PERCENTAGE FREQUENCIES OF THE VARIOUS FORMS WITH RESPECT TO LENGTH OF PIECE AND BODY LEVEL IN BUDDING ANIMALS

| Body Length Divided In | Body Level | No. of Pieces | Unipolar | Bipolar | Multipolar | Budding | No Reconstitution or Death |
|---|---|---|---|---|---|---|---|
| Fourths | 1 | 21 | 33 | 30 | 37 | ..... | ..... |
| | 2 | 21 | 48 | 14 | 20 | ..... | 18 |
| | 3 | 21 | 48 | 14 | 25 | ..... | 13 |
| | 4 | 21 | 95 | ..... | 5 | ..... | ..... |
| Sixths | 1 | 44 | 25 | 27 | 48 | ..... | ..... |
| | 2 | 44 | 38 | 11 | 51 | ..... | ..... |
| | 3 | 44 | 43 | 11 | 41 | 5 | ..... |
| | 4 | 44 | 41 | ..... | 45 | ..... | 14 |
| | 5 | 43 | 53 | ..... | 28 | 5 | 14 |
| | 6 | 43 | 40 | ..... | ..... | 42 | 18 |

Very little mention of depression is made by American investigators until recently, when apparently Kepner and Jester (1927) have seen and described this phenomenon for *Chlorhydra viridissima* (Schulze) Pallas (=*Hydra viridis* Linnaeus). Depression has been discussed both historically and experimentally in a recent paper by Hyman (1927). Briefly, depression is a physiological condition in hydra which is first recognized by the refusal of the animal to feed, followed by a contraction of the stalk and body and gradual resorption of the tentacles. The tentacles soon disappear completely or more stumps remain, and the animal no longer holds itself erect but lies on the substratum. The color of the animal changes from a pronounced brown to a dirty yellow. Various theories, which have been summarized by Hyman, have been offered to account for depression,

but whatever may be the environmental factor or factors concerned, a condition of low metabolism apparently exists in the animals, as indicated by the susceptibility to cyanide (Hyman, 1927). The writer has been able to induce depression by the exposure of the animals to different concentration of KCN for varying intervals of time. This, when followed by recovery, has given rise to various abnormalities such as branched tentacles, double hydranths, etc., which have been described many times since Trembley's first observations. A complete presentation of these data is reserved for a later paper.

The hydras used for these experiments had undergone depression under natural conditions in the cultures. They were of as large size as possible, but showed no visible indications of budding.

Most of them had completely absorbed the tentacles, and the hypostome was poorly differentiated. The animals, which were as nearly uniform in size as possible and in the same general stage of depression, were selected in groups of five or six at varying intervals over a period of several weeks from different cultures and sectioned. In sectioning the animals, the apical piece, which would be equivalent to the hypostome and tentacles of a normal specimen, was removed and the body region then divided into the number of pieces desired by the usual method. Reconstitution of pieces of the body region of animals divided into thirds, fourths, and sixths was studied. These data are summarized in Table III.

It will be noted that the number of pieces for the various levels is not so large in Table III as in former experiments. In view of the striking uniformity in results obtained and the random selection of the animals for these experiments at various times, the data seemed sufficient. Table III compared with the thirds, fourths, and sixths of Table I shows a marked decrease in multipolar frequency greatest at the more distal levels, and a marked decrease in bipolar frequency, apparently general. There is a marked increase in unipolar frequency greatest at the more distal levels. In piece one of the sixths, the unipolar increase is 82 per cent. The form frequencies of unipolar and multipolar forms in sixths from depressed animals as given in Table III are shown in Graph 1 by the curves lettered *D*. The frequency differences between the pieces of animals in good

condition (Table I) and the depressed animals (Table III) can be readily seen by comparing the curves lettered *N* and *D*, respectively, in the graph. The unbroken line *D* compared to the unbroken line *N* shows that the frequency of unipolar forms in the depressed pieces is much higher at all levels than in pieces in good condition. This difference is most marked in the distal levels, particularly level 1, where there is a frequency difference of 82 per cent. Comparison of the broken line *D* and the dotted line *D* with the correspond-

TABLE III

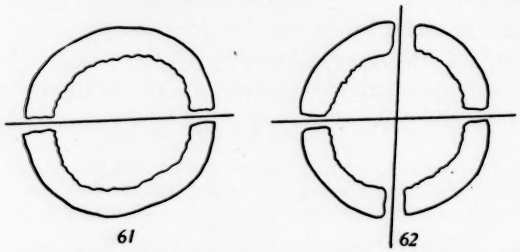
PERCENTAGE FREQUENCIES OF THE VARIOUS FORMS WITH RESPECT TO LENGTH OF PIECE AND BODY LEVEL IN DEPRESSED, NON-BUDDING ANIMALS

| Body Length Divided In | Body Level | No. of Pieces | Unipolar | Bipolar | Multipolar | Budding | No Reconstitution or Death |
|---|---|---|---|---|---|---|---|
| Thirds | 1 | 22 | 70 | 9 | 11 | ..... | 10 |
| | 2 | 22 | 41 | ..... | ..... | ..... | 59 |
| | 3 | 22 | 54 | ..... | ..... | 10 | 36 |
| Fourths | 1 | 26 | 80 | 15 | 5 | ..... | ..... |
| | 2 | 26 | 65 | ..... | 4 | 27 | 4 |
| | 3 | 26 | 73 | 4 | 4 | 19 | ..... |
| | 4 | 26 | 57 | ..... | ..... | 39 | 4 |
| Sixths | 1 | 10 | 100 | ..... | ..... | ..... | ..... |
| | 2 | 10 | 60 | 10 | ..... | ..... | 30 |
| | 3 | 10 | 90 | ..... | ..... | ..... | 10 |
| | 4 | 10 | 80 | ..... | 10 | ..... | 10 |
| | 5 | 9 | 78 | ..... | 11 | 11 | ..... |
| | 6 | 9 | 68 | ..... | ..... | 34 | ..... |

ing lines *N* shows that the frequencies of bipolar and multipolar forms in depressed pieces are much lower than in pieces in good condition, and that the decrease is greatest at the more distal levels. This increase in unipolar forms is the more striking when the relatively high percentage of pieces either budding or showing no reconstitution or death is taken into consideration. The high percentage of budding in these depressed specimens is itself of interest, since it is much higher in the more proximal levels for the fourths and sixths than it is in normal, non-depressed specimens. It is also interesting to note that in the fourths budding occurred in the entire proximal three-fourths of the body, an extension of the budding zone far beyond its limits in normal animals.

FORM FREQUENCIES IN VERY SMALL PIECES

It is difficult to cut pieces shorter than one-tenth of the body length, but pieces of smaller volume can be obtained by bisecting and quadrisecting transverse sections of the body as indicated in




FIGS. 61–62.—Diagrams showing the method of cutting "bisected" and "quadrisected" body sections.

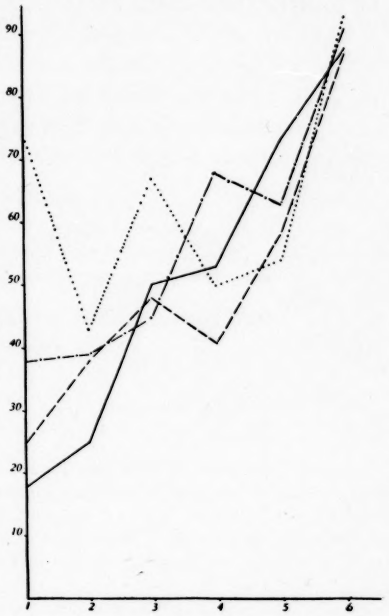
Figures 61 and 62. These small pieces of the bisected and quadrisected circles of tissue soon fuse at the lateral cut ends and thus form small circles of tissue. This was shown by Weimer (1927) in a previous paper. Table IV gives the frequencies for bisected and quadrisected sixths of budding animals. The volume of the bisected sixths is, of course, only slightly less than that of the tenths of the body length, but that of the quadrisected sixths is considerably less.

TABLE IV

PERCENTAGE FREQUENCIES OF THE VARIOUS FORMS WITH RESPECT TO LENGTH AND DIAMETER OF PIECE AND BODY LEVEL IN BUDDING ANIMALS

| Body Length Divided In | Body Level | No. of Pieces | Unipolar | Bipolar | Multipolar | Budding | No Reconstitution or Death |
|---|---|---|---|---|---|---|---|
| Sixths (bisected) | 1 | 44 | 38 | 48 | 14 | ..... | ..... |
| | 2 | 46 | 39 | 33 | 26 | ..... | 2 |
| | 3 | 39 | 46 | 31 | 21 | ..... | 2 |
| | 4 | 46 | 63 | 11 | 21 | 5 | ..... |
| | 5 | 44 | 52 | 14 | 23 | 11 | ..... |
| | 6 | 44 | 57 | ..... | 9 | 34 | ..... |
| Sixths (quadrisected) | 1 | 48 | 73 | 20 | 2 | ..... | 5 |
| | 2 | 48 | 43 | 48 | 9 | ..... | ..... |
| | 3 | 48 | 67 | 14 | 19 | ..... | ..... |
| | 4 | 48 | 50 | 21 | 27 | ..... | 2 |
| | 5 | 48 | 54 | 22 | 24 | ..... | ..... |
| | 6 | 48 | 75 | ..... | 6 | 19 | ..... |

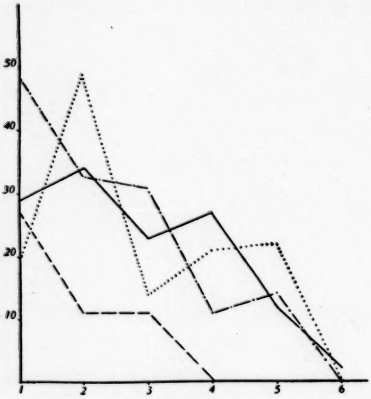
A comparison of Table IV with the sixths of Table II, also from budding animals, shows as the most conspicuous feature of Table IV a decrease in multipolar frequency greatest at the more distal levels and distinctly greater in the quadrisected than in the bisected pieces. There is also an increase in bipolar frequency, though with no definite relation to level and about the same in bisected and quadrisected pieces. Finally, there is a probable increase in unipolar frequency in the bisected pieces without definite relation to level and a marked increase in the quadrisected pieces apparently greatest in the distal one-third, where unipolar frequency is lowest in the larger pieces.



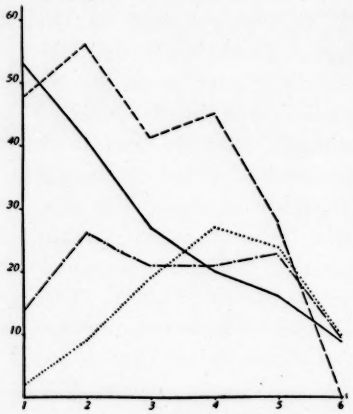

GRAPH 2.—Graph showing the frequencies of the unipolar forms in *P. oligactis* from the data on sixths in Tables I and II and the bisected and quadrisected sixths in Table IV. The ordinates represent the percentage frequencies of the unipolar forms and the abscissae the body levels of the animal, 1 representing the most apical. The solid line ——— shows the frequencies of unipolar forms from the sixths of non-budding animals; the broken line – – – –, sixths for budding animals; the dot-dash line .—.—., bisected sixths of budding animals; and the dotted line .....:....., quadrisected sixths of budding animals.

Graph 2 shows the frequencies of the unipolar, Graph 3 of the bipolar, and Graph 4 of the multipolar forms in sixths of non-budding animals (Table I), sixths of budding animals (Table II), and bisected and quadrisected sixths (Table IV). A comparison of Graphs 2, 3, and 4 shows that the unipolar forms increase (Graph 2) and bipolar (Graph 3) and multipolar (Graph 4) decrease in the proximal direction. In Graph 2 there is little difference between the frequencies for the non-budding (unbroken line), budding (broken line), and bisected sixths (dot-dash line), but quadrisected

sixths (dotted line) show more increase at the more distal levels. According to Graph 3, no very clearly marked differences in the fre-




GRAPH 3




GRAPH 4

GRAPH 3.—Graph showing the frequencies of the bipolar forms in *P. oligactis* from the data on sixths in Tables I and II and the bisected and quadrisected sixths in Table IV. The ordinates represent the percentage frequencies of bipolar forms and the abscissae the body levels of the animal, 1 representing the most apical. The solid line ——— shows the frequencies of the bipolar forms from the sixths for non-budding animals; the broken line – – – –, sixths for budding animals; the dot-dash line .—.—.—., bisected sixths of budding animals; and the dotted line ....., quadrisected sixths of budding animals.

GRAPH 4.—Graph showing the frequencies of the multipolar forms in *P. oligactis* from the data on sixths in Tables I and II and the bisected and quadrisected sixths in Table IV. The ordinates represent the percentage frequencies of the multipolar forms and the abscissae the body levels of the animal, 1 representing the most apical. The solid line ——— shows the frequencies of the multipolar forms from the sixths of non-budding animals; the broken line – – – – –, sixths for budding animals; the dot-dash line .—.—.—., bisected sixths of budding animals; and the dotted line ....., quadrisected sixths of budding animals.

quencies of the bipolar forms appear under the different conditions. Graph 4, however, seems to show a considerable decrease in multipolar frequency in bisected (dot-dash line) and quadrisected (dotted line) at the more distal levels. The irregularity of these curves is due undoubtedly to the variations in the size of these small pieces and to the small number of pieces cut.

### FREQUENCIES OF THE VARIOUS FORMS IN RELATION TO SIZE OF PIECE

In order to see more clearly the effect of the size of the piece on the frequencies of the various forms irrespective of body level, it seemed desirable to summarize the data which have been previously presented in Tables I, II, III, and IV from this viewpoint. This has been done in Table V. According to this table, there is no consistent relation between the form frequencies and length and size of the

TABLE V

TOTAL FREQUENCIES OF THE VARIOUS FORMS AS PERCENTAGES IN RELATION TO LENGTH OF PIECE IRRESPECTIVE OF BODY LEVEL

| Condition of Animal | Length of Piece | No. of Pieces | Unipolar | Bipolar | Multipolar | Budding | No Reconstitution or Death |
|---|---|---|---|---|---|---|---|
| Non-budding | Entire | ..... | 100 | ..... | ..... | ..... | ..... |
| | Halves | 50 | 63 | ..... | 18 | 19 | ..... |
| | Thirds | 135 | 52 | 7 | 17 | 15 | 11 |
| | Fourths | 264 | 60 | 12 | 23 | 3 | 2 |
| | Sixths | 262 | 49 | 21 | 27 | 2 | 1 |
| | Tenths | 332 | 62 | 21 | 15 | .5 | 1.5 |
| Budding | Fourths | 84 | 56 | 14 | 23 | ..... | 7 |
| | Sixths | 262 | 40 | 8 | 35 | 9 | 8 |
| Non-budding in depression | Thirds | 66 | 55 | 3 | 4 | 3 | 35 |
| | Fourths | 104 | 69 | 5 | 3 | 21 | 2 |
| | Sixths | 58 | 79 | 1.5 | 4 | 7 | 7.5 |
| Budding | Sixths bisected | 263 | 49 | 23 | 19.5 | 8 | .5 |
| | Sixths quadrisected | 288 | 80 | 21 | 16.5 | 3 | 1.5 |

piece, or if any such relation exists it is obscured by the occurrence of budding and the failure of some of the budding pieces to reconstitute. In the depressed pieces of Table V unipolar frequency apparently increases with decrease in length of the piece, but the high frequency of failure to reconstitute or of budding in the thirds and fourths of this series makes it doubtful whether this apparent increase has any significance.

In the non-budding animals from the halves to and including the sixths the frequency of bipolar and multipolar forms appears to increase. This increase is correlated with a decrease in the frequency of budding and failure to reconstitute with decreasing length of the

piece. In the budding animals the frequency of the multipolar forms is 12 per cent higher in the sixths than in the fourths, but the frequency of budding also increases in the sixths. In the pieces from depressed animals few bipolar and multipolar forms occur, and the frequency of budding or failure to reconstitute is high in the thirds and fourths. In the bisected and quadrisected sixths, bipolar and multipolar frequencies differ only slightly. Within the limits of size in these experiments, i.e., from halves to quadrisected sixths, it appears that length or size of piece is not a primary factor in determining the total frequencies of the various forms at all body levels taken together, but Tables I–IV and Graphs 1, 2, 3, and 4 show that these frequencies are related to body level.

### RATE OF TENTACLE FORMATION IN RELATION TO BODY LEVEL

According to the theory of Child (1915, 1924), the axiate pattern of the organism in its simplest form consists in quantitative differences in physiological condition at various levels which constitute a gradient or gradients in the protoplasm. Those regions with high metabolic activity reconstitute and differentiate more rapidly than those with lower activity. Similarly, animals in which a high rate of metabolism prevails will reconstitute and regulate more rapidly than those with a lower rate. The existence of such relations apparently has been demonstrated by Child, Hyman, and others for many animals. These same workers have accumulated a mass of evidence on the gradient relations in the Coelenterata, particularly for *Tubularia*, *Corymorpha*, and some of the actinians.

The existence of susceptibility gradients in *P. oligactis* has been shown by Child and Hyman (1919) with the use of various concentrations of potassium cyanide, ethyl alcohol, ethyl ether, and various dyes, and by Hinrichs (1924) with ultra-violet light. The following data are of interest since they further confirm the observations previously made on hydra.

Child (1915, 1924) has shown that the frequencies and the rate of reconstitution of the different head forms in *Planaria* are apparently associated with quantitative differences in metabolic rate at different body levels. A relation between length of hydranth primordium and rate of reconstitution has been reported for *Tubularia*

by Driesch (1899), Morgan (1901*a*, 1901*b*, 1906, 1908), Child (1907), Hyman (1920, 1926), and by Child and Hyman (1926) for *Corymorpha*. These observations were made on the time required for hydranth formation. It seemed possible, therefore, that some indication of the gradient relations in hydra might appear in the time required for tentacles to form on pieces from different body levels. Since the tentacle must be regarded as a typical apical structure, theoretically these should appear most rapidly both from the standpoint of time and of number in those regions where metabolic activity is greatest. Accordingly, in a number of cases accurate records were kept of the total number of tentacles counted each day on the various pieces from different levels of the body. No attempt was made to measure the rate of growth in length of the tentacle. No structure was recorded as a tentacle until its nature was unmistakably evident.

It has been previously noted that a reconstituting piece may have more than one axis, as is indicated by the formation of perfect or partial hydranths in different regions. However, in almost every case there is one axis which seems to be more pronounced than the others in that its apical stuctures are better differentiated. In order to make the tentacle numbers of the various forms at different levels comparable to one another, as, for example, unipolar forms and bipolar-unipolar forms, the following plan was used. The total tentacle number of all the pieces at a given level on a certain day is expressed as a fraction, the denominator of which indicates the total number of tentacles on a piece irrespective of their position, or, in other words, of the number of axes, and the numerator shows the number of tentacles present on the apical end of the major axes of the pieces under consideration. Thus, for example, if the tentacle number of the pieces shown in Figures 3, 20, and 50 were recorded, it would be expressed as 11/17. In all cases a branched tentacle is counted as one. Tentacles on buds of budding pieces are not counted.

Table VI shows the number of tentacles on pieces from various body levels of *P. oligactis* in good condition from the second to the fifth day following cutting, no tentacles having appeared during the first day. The first three columns have the same meaning as in pre-

vious tables. The four following columns show the total number of tentacles counted each day from the second to the fifth day following section for all pieces of a certain level. One unfortunate feature of Table VI is that the number of pieces is not uniform for different levels. In Table VI the number of tentacles formed by the pieces at the various levels can be made approximately comparable by multiplying the daily tentacle number of thirds and sixths by 2.

Table VII shows the number of tentacles on pieces from various body levels of depressed *P. oligactis* each day from the second to the

TABLE VI

TOTAL DAILY TENTACLE NUMBERS ON PIECES OF VARIOUS LENGTH FROM DIFFERENT BODY LEVELS OF NON-DEPRESSED ANIMALS

| BODY LENGTH DIVIDED IN | BODY LEVEL | NO. OF PIECES | DAYS | | | |
|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 |
| Thirds..... | 1 | 21 | 49/74 | 63/77 | 71/92 | 78/101 |
| | 2 | 21 | 5 | 50/55 | 67/77 | 75/81 |
| | 3 | 21 | 14 | 58 | 61/64 | 71/76 |
| Fourths..... | 1 | 42 | 68/91 | 92/130 | 119/156 | 120/163 |
| | 2 | 42 | 26/31 | 69/89 | 85/106 | 91/109 |
| | 3 | 42 | 13/14 | 77/88 | 100/113 | 110/123 |
| | 4 | 42 | 39/40 | 117/122 | 137/144 | 128/135 |
| Sixths..... | 1 | 22 | 31/35 | 48/70 | 52/78 | 55/80 |
| | 2 | 22 | 11/12 | 41/66 | 41/66 | 49/67 |
| | 3 | 22 | 5 | 37/49 | 39/51 | 43/55 |
| | 4 | 22 | 7 | 36/47 | 39/43 | 56/62 |
| | 5 | 22 | 0 | 23/25 | 42/37 | 48/54 |
| | 6 | 22 | 3 | 44/46 | 53/56 | 56/59 |

fifth following cutting. Here again there is a variable number of pieces for different levels, especially the thirds and sixths. In order to make Table VII approximately comparable with Table VI, the numbers of tentacles which would have formed on numbers of pieces corresponding to those of Table VI are calculated from the number of tentacles observed and the number of pieces used. The numbers in parentheses in column 3 of Table VII indicate the number of pieces actually observed. In the proximal thirds of Table VI three pieces budded on the third and five on the fourth day, and in Table VII one piece budded on the second day. Since tentacle numbers of these pieces were not included in the numbers tabulated, these are somewhat lower than if budding had not occurred.

Tables VI and VII both show with few exceptions, first, that the numbers of tentacles at any given time and the total number of tentacles found are greater in the distal and proximal than in the middle body regions; second, the distal pieces give rise to the greatest total number of tentacles, the proximal pieces are next, and the pieces from the middle regions show the smallest numbers; third, on the second day the distal pieces have more tentacles than the proximal pieces on the major axes, but later the numbers on the

TABLE VII

TOTAL DAILY TENTACLE NUMBERS ON PIECES OF VARIOUS LENGTH FROM DIFFERENT BODY LEVELS OF ANIMALS IN DEPRESSION

| BODY LENGTH DIVIDED IN | BODY LEVEL | NO. OF PIECES | DAYS | | | |
|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 |
| Thirds..... | 1 | 22 | 23 | 33/35 | 34/39 | 29/32 |
| | 2 | 22 | 4 | 10 | 13 | X |
| | 3 | 22 | 15 | 17 | 27 | X |
| Fourths..... | 1 | 42 (26) | 26/27 | 46/55 | 61/80 | 84/98 |
| | 2 | 42 (26) | 11/12 | 26/30 | 32/35 | 43/47 |
| | 3 | 42 (26) | 11 | 37/41 | 42 | 60 |
| | 4 | 42 (26) | 32 | 63 | 75 | 92 |
| Sixths..... | 1 | 22 (10) | 15 | 40 | 46 | 55 |
| | 2 | 22 (10) | 2 | 2 | 11 | 15 |
| | 3 | 22 (10) | 0 | 13 | 18 | 31 |
| | 4 | 22 (10) | 4 | 4 | 20/22 | 35/36 |
| | 5 | 22 (10) | 0 | 20/22 | 20/22 | 33/35 |
| | 6 | 22 (10) | 0 | 24 | 33 | 33 |

major axes of the proximal pieces approach, equal, or exceed those of the distal.

Comparison of the corresponding levels of Tables VI and VII for corresponding days shows that the tentacle numbers on pieces in good condition are greater than in the depressed pieces—often twice as great. In other words, the tentacles appear more slowly on the depressed pieces.

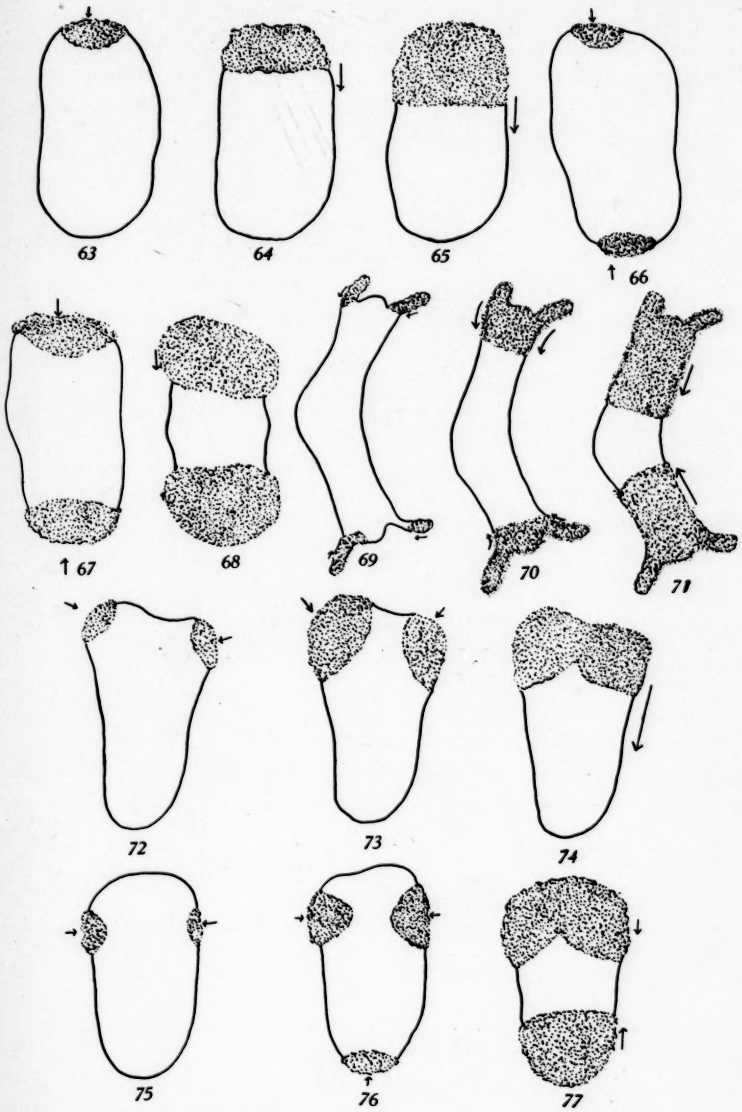
## SUSCEPTIBILITY GRADIENTS IN EARLY STAGES OF RECONSTITUTION

If the axis is a gradient it should be possible to demonstrate its presence even before apical differentiation has occurred. The following data on susceptibility are suggestive in this connection, but,

since the pieces used for the determination of susceptibility do not develop further, these data are not demonstrative except when apical structures are already present.

The body regions of non-budding animals were divided into fourths and placed in prepared well water for twenty-four hours to allow the cut ends to heal and the stimulus of cutting to subside. The pieces were then placed in a solution of $M/300$ KCN made up in prepared well water, and the course of disintegration was followed. In Figures 63–65 a piece is shown which was completely disintegrated by only one disintegration wave. Here, apparently, there was only one locus of high rate, and, supposedly, the piece was an incipient unipolar form. Figures 66–68 show progressive stages in the disintegration of a piece with two loci of high susceptibility. These appeared to be of equal susceptibility, for disintegration began simultaneously at both. Supposedly this piece, if allowed to reconstitute, would have been a bipolar form. Comparing Figures 66–68 with Figures 69–71, which show the gradients in the later stages of a bipolar form, it is found that the gradients as indicated by susceptibility are similar in both. Figures 72–74 show the disintegration of a piece which probably would have given rise to a typical multipolar form. Two waves of disintegration spread from two areas at one end of the piece. These approached, the one wave slightly in advance of the other, met, and traversed the rest of the piece as one. Figures 75–77 show the gradient relations in an apparently incipient multipolar form. In this piece areas of disintegration appeared simultaneously opposite each other near one end of the piece. After these had progressed for a little distance, a third area appeared at the other end. The two areas at one end met, fused, and advanced down the piece to meet the disintegration wave coming from the other end.

These figures by no means exhaust the variations in susceptibility. In some cases one disintegration wave may traverse almost the whole piece and then one or more small second areas may appear. Sometimes the wave will start obliquely across the piece and travel some distance in this manner before becoming transverse to the long axis of the piece. However, in general the various patterns of disintegration observed are but variations of the four types just de-




FIGS. 63–77.—Determination of axes of reconstituted pieces by direct susceptibility to KCN. Figs. 63–65 show a probable unipolar form; Figs. 66–71, bipolar forms; Figs. 72–74, a probable multipolar form of the bipolar-unipolar type; Figs. 75–77, a probable multipolar form of the multiapical type.

scribed. If these data can be trusted, it appears that the apical ends of new polarities originate at various loci of high susceptibility and so presumably of high rate of metabolism. Under the usual conditions these loci, and hence presumably the form of the reconstituted piece, are established within twenty-four hours after cutting.

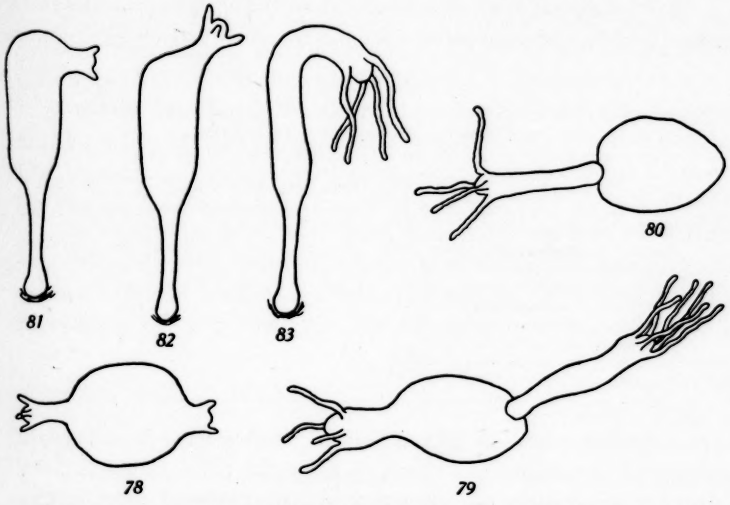
### EXPERIMENTS ON BUDDING

According to the gradient theory, the apical region is dominant over other body levels within a certain distance. Physiological isolation may result from increase in length of body, decrease in dominance, blocking of the process of control, or direct acceleration of the subordinate region. Physical isolation results from the removal of the dominant region. The budding zone of (*Pelmatohydra*) apparently represents the most proximal region capable of giving rise to a new individual, since the stalk is apparently incapable of such reorganization. Budding, interpreted in terms of the gradient theory, results from some degree of physiological or physical isolation from the dominance of the apical region.

The following experiments concern the effect on budding and separation of buds of physical isolation of parts of the body including the budding zone. In pieces from the budding zone new apical ends may arise from either one or both cut ends of the piece, or they may arise as buds from the lateral region. But the fate of the lateral bud may differ according to conditions. The bud may continue its development and separate (Figs. 78–80) as buds normally do, or it may attain dominance over the piece, become its apical end, and remain permanently a part of it (Figs. 81–83). As in all experiments on non-depressed animals, the largest individuals without buds in the cultures were used. Such animals are almost ready to bud, and some degree of physiological isolation and consequent increase in activity has probably already taken place in the budding zone. That such is the case is indicated by the experiments on the rate of tentacle formation, which shows that pieces from the budding zone develop more tentacles in a given time than the middle body regions (Tables VI and VII). The animals were cut as desired, the hypostome and tentacles being removed in all cases while the stalk was removed in some lots and remained in others. A total of twenty

animals in lots varying in number was selected at different times and used as controls. These hydras were placed in stender dishes under the same conditions as the cut pieces.

The data on budding and bud separation for the non-depressed animals are summarized in Table VIII. In all experiments with pieces less than the entire body length, the proximal piece of the




FIGS. 78–80.—Bud formation and separation from a piece of the body region from the budding zone, following removal of distal body region and stalk.

FIGS. 81–83.—Bud formation and control of parent-piece following removal of distal body region only.

body was used, since the budding zone is in this proximal region. The first column indicates that the pieces are proximal and also shows whether the stalk was present or removed; the second column, the size of the body piece; the third column, the number of animals; the fourth, the number of pieces in percentages giving rise to well-defined buds which separate from the parent-animal. The percentage of budding as shown by bud separation for pieces other than those from the most proximal level is so small that only the latter have been considered in this table. However, it is worth noting that in pieces of depressed animals the budding zone may extend to more

distal levels (p. 203). A study of Table VIII shows that budding followed by bud separation occurs most often in pieces consisting of the proximal one-half to one-third of the body piece from which the stalk has been removed. If the stalk remains on the piece the percentage of bud separation is greatly lowered or reduced to zero.

## DISCUSSION

Many different lines of evidence from the work of many investigators indicate or demonstrate the existence of physiological gradi-

TABLE VIII

FREQUENCY OF BUD SEPARATION IN PIECES OF VARIOUS SIZES FOLLOWING THE REMOVAL OF MORE APICAL LEVELS

| Body Level | Size of Piece | No. of Animals | Per Cent of Bud Separation |
|---|---|---|---|
| Entire: stalk present | ..... | 20 | 0 |
| Entire: stalk removed | ..... | 35 | 6 |
| Proximal: stalk present | $\frac{1}{2}$ | 6 | 0 |
| Proximal: stalk removed | $\frac{1}{2}$ | 17 | 35 |
| Proximal: stalk removed | $\frac{1}{3}$ | 45 | 44 |
| Proximal: stalk removed | $\frac{1}{4}$ | 66 | 9 |
| Proximal: stalk removed | $\frac{1}{6}$ | 44 | 8 |
| Proximal: stalk removed | $\frac{1}{10}$ | 22 | 0 |
| Control | ..... | 20 | 4 |

ents in Coelenterata other than hydra. This evidence includes data on rate of reconstitution; length of hydranth primordium and frequency of different forms; differences in oxygen consumption, in $CO_2$ production, in oxidation-reduction reactions, in electric potential, and in susceptibility in relation to level of the body. It concerns *Tubularia*, *Corymorpha*, *Obelia*, and various other hydroids, several medusae, and some actinians.[1]

[1] The more important literature bearing on the gradient problem in Coelenterates is as follows. On *Tubularia*, Driesch (1899), Morgan (1901*a*, 1901*b*, 1902, 1903, 1906, 1908), Child (1907*a*, 1907*b*), and Hyman (1920) studied the rate of reconstitution, length of hydranth primordium, and frequency of bipolar and unipolar forms in relation to level; Hyman (1926), oxygen consumption; Hyman and Bellamy (1922), electric potential differences. Child (1926, 1927) and Child and Hyman (1926) investigated differential susceptibility, oxygen consumption, $CO_2$ production, oxidation-reduction reactions, electric potential, rate of reconstitution, frequencies of different forms in relation to level, and modifications of polarity in reconstitution in *Corymorpha*. As regards other hydroids, Mathews (1903) and Lund (1921, 1922, 1923) worked on potential differences; Gast and Godlewski (1903), rate of reconstitution; Child (1919,

Indirect evidence for the existence of gradients in hydra is afforded by the early experiments on grafting. Rand (1898*a*–*b*), Hefferan (1902), and King (1901, 1903) found as a result of their experiments on grafting hydra that whether a graft would organize a new apical structure on the host or be absorbed or fail to persist depended for the most part on the level of the body represented by the graft tissue and the region of the host on which the graft was made. In general, grafts from the distal levels of one hydra to the proximal levels of another showed a tendency to persist and form hydranths, while grafts from proximal into distal regions either failed to persist or were absorbed. Browne (1909) and Koelitz (1910) found that a portion of the peristome with or without a tentacle if grafted into the host in any region other than the most distal will organize the host tissue into either a hydranth or a new hydra, depending on the distance of the graft from the apical end of the host. If grafted into the most distal region, the tissue is absorbed. The experiments of Issajew (1926) and other data obtained by the writer but not reported in this paper indicate that the body pieces of hydra which have a relatively high metabolic rate become dominant apical regions and organize the surrounding tissue of lower rate irrespective of the body level, provided there is sufficient difference in rate.

Rand (1911) concluded from the experiments on grafting that in the normal adult hydra the peristome, whether in normal position or transplanted, is the seat of some sort of control over the column substance. In the light of the latter experiments it may be concluded that in general pieces of hydra with a sufficiently high rate of metabolism when brought into organic connection with regions of a sufficiently lower rate become centers of organization and apical ends of new polarities.

*Size of piece and level of the body in relation to reconstitution.*—These experiments show that reconstituting pieces from the body region of budding and non-budding *P. oligactis* give rise to a variety of forms which may be grouped according to the number of axes

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1921, 1923, 1925), susceptibility and reduction in relation to level; and Hyman and Bellamy (1922), potential differences in medusae. Child (1910) recorded data on bipolar and unipolar forms in relation to body level in actinians.

present into three classes; unipolar, bipolar, and multipolar. This general similarity between the budding and the non-budding hydras is probably due to the fact that the non-budding animals are on the verge of budding and hence are little different physiologically from the budding animals. The frequency of these forms depends on the size of the piece, the level of the body, and the general physiological condition of the animal (Tables I–IV). In general, in the normal animals with decrease in size of the piece to one-half and one-third of the body length bipolar and multipolar forms appear, and with further decrease in size the frequency of these forms increases in the more distal regions with, however, no definite increase in total frequency. Except in the halves, the frequency of the unipolar forms is in general lowest at the distal levels and increases proximally in pieces of a given length, but there is no evident relation between unipolar frequency and length of the piece within the limits of the tables. In pieces from depressed as compared with those from normal animals, there is a marked decrease in multipolar forms in the more distal levels, a marked decrease of bipolar forms throughout, and a marked increase in unipolar forms, greatest at the more distal levels.

Unipolar forms represent of course a single polarity, which may conceivably coincide in direction with the original gradient, be opposite in direction to it or in some other direction. Probably, at least in the longer pieces, the gradient of unipolar forms coincides in direction with the original gradient. Whether, or to what extent, a polarity may be determined in pieces of hydra by the differential of position, i.e., by the differences in condition between the free surface and the surface in contact as the piece lies on the bottom, is very difficult to determine, since no entirely satisfactory method has been devised for marking small pieces in such manner that the direction of the original axis can be distinguished during reconstitution, though various attempts in this direction have been made. Attempts have also been made to hold pieces with a certain region in contact, but without marked success, though some of the results obtained were suggestive. At present it is not certain what relation or relations, as regards direction, the polarity of unipolar forms may have to the original gradient.

In pieces from animals in good condition, unipolar frequency is lowest at the distal levels and increases proximally. In pieces from depressed animals it is higher at all levels than in those in good condition, but the greatest increase occurs in the distal pieces. Also in the bisected and quadrisected sixths it is distinctly higher in the distal region than in the larger pieces in good condition. Depression and decrease in size of piece below a certain limit increase unipolar frequency at those body levels where it is normally lowest. Depression is apparently associated with low metabolism, and pieces of depressed animals show less contractile activity and change of position than do those in good condition. This suggests the possibility that the differential of position may be concerned in determining the increase in unipolar frequency in the distal pieces of depressed animals. Also the lower metabolic activity of such pieces should permit the obliteration of the original gradient fraction by the differential of position more frequently than in pieces in good condition.

It is conceivable that the increase in unipolar frequency from distal to proximal levels may likewise be the result of an increasing effect of the differential of position, either because the motor activity of the more proximal is less than that of the distal pieces or because the gradient differences are less and therefore more readily obliterated in the proximal than in the distal pieces, or both factors may be concerned. The indications from the data on tentacle formation that the budding zone is more active than the middle body region afford some basis for the belief that the gradient differences in pieces of a given length are less marked in the proximal than in the distal regions.

In the bisected and quadrisected sixths the unipolar frequency at distal levels is also higher than in larger pieces in good condition. It is possible that in these very small pieces with little of the original gradient present the differential of position is more effective in determining polarity than in larger pieces.

In the shorter pieces of *Tubularia* the frequency of unipolar forms increases from distal to proximal levels (Driesch, 1899; Morgan, 1901, 1902, 1906, 1908; Child, 1097*a*, 1907*b*). In the actinian *Harenactis* (Child, 1910) bipolar forms occur only at the more distal levels. In these cases the difference is quite independent of the dif-

ferential of position and apparently due to some internal factor, perhaps to steepness of the gradient.

In *Corymorpha* a similar increase in unipolar frequency from distal to proximal levels has been attributed by Child (1926) to the fact that the more proximal pieces, having a larger diameter in proportion to their length, stand on end more frequently. This interpretation will not serve for hydra, since no marked difference in diameter exists at different levels of the body proper. In view of all these facts, the possibility that the increase in unipolar frequency from distal to proximal levels in pieces in good condition is due solely to some internal gradient factor not yet recognized cannot be ignored. If this is the case, however, it becomes more difficult to account for the increase in unipolar frequency at the more distal levels in depressed and the very small pieces.

But whatever the factors involved in determining the polarity of unipolar forms, it is evident that a relation between their frequency and the original gradient of the animal exists and that this relation is altered by change in physiological and experimental conditions.

Bipolar forms resulting from reconstitution of pieces have been described by various investigators for a number of hydroids, but so far as the writer has been able to determine from the literature, not for hydra. Wetzel (1895), King (1903), Browne (1909), and Koelitz (1910) have been able to reverse the polarity in pieces of hydra by grafting. Koelitz has concluded as a result of these experiments that heteromorphosis in hydra can be brought about only as a result of grafting. Goetsch (1920) describes a bipolar animal which was formed by a bud taking control of each cut end of the parent-piece, and concludes later (Goetsch, 1925) that in regeneration in hydra pieces always retain their original polarity.

The results of these experiments show that the production of bipolar (biaxial, heteromorphic) forms, involving a reversal of polarity in one or more regions of the reconstituting piece, can be brought about by methods other than grafting or budding. The production of a bipolar form ("axial heteromorphosis") in *P. oligactis* depends apparently upon the size of the piece, the level of the body from

which the piece is taken, and, most of all, the gradient relations in that piece. The bipolar forms in the hydroids represent a persistence of the original gradient distally and its obliteration by a new gradient in the opposite direction proximally. This new proximal gradient is determined by conditions at the proximal cut end. The bipolar forms in hydra appear to be similar in character and determined in the same way. Pieces comprising the entire body and halves do not give rise to bipolar forms. In such pieces the original gradient direction persists unchanged. With decrease in length of piece to one-third, bipolar forms appear, and with further decrease in length their frequencies increase at the more distal levels, but without further increase in total frequency (Table I).

Bipolar forms appear only in pieces of hydra below a certain length, i.e., the differences in condition at the two ends must not be too great, otherwise the more active end will dominate the other and determine its development as a proximal region. Their higher frequency at the more distal levels may conceivably result from a greater motor activity of the more distal pieces and more frequent change of position. Equal exposure of the two ends will be more frequently attained in more active than in less active pieces. It has been shown that *Corymorpha* pieces moved about and turned over give a higher frequency of bipolar forms than those allowed to lie undisturbed on the bottom (Child, 1926). On the other hand, the frequency distribution of the bipolar forms in hydra may be dependent on gradient factors which are not yet recognized.

The multipolar forms are not a homogeneous group. Some bipolar-unipolar forms may arise by the development of a new axis on some lateral region of the stem which develops as a proximal structure because of the inhibiting action of the two apical regions (Figs. 30, 36, 38). These forms are modifications of bipolar forms. The other type of bipolar-unipolar form is very clearly a result of the presence of more than one growing region at one cut surface, probably the distal (Figs. 29, 31–34). In such cases the gradient of the piece is not sufficiently altered by sectioning to permit a new dominant region to arise at the proximal end, consequently no apical structures are produced there. A third kind of multipolar form, the

multiapical (Figs. 41–57), possesses three or four apical ends. Such forms may involve the appearance of adventitious polarities independent of the original gradient and the cut ends.

The frequency distribution of the multipolar forms in relation to level is in general similar to that of the bipolar forms, i.e., they occur more frequently at the more distal levels. As Table I shows, they occur in halves, but in such cases they are bipolar-unipolar forms with two apical regions developing from one cut end. Such forms may occur in pieces of any length, but the other types are more frequent in shorter pieces. Since this group is heterogeneous in character, conditions determining frequency may differ for the different forms. At present it can only be noted that its distribution is in general similar to that of the bipolar forms.

Evidently the conditions determining the frequencies of unipolar forms, on the one hand, and bipolar and multipolar forms, on the other, are to some extent mutually exclusive or antagonistic, for we find that as the unipolar forms increase, bipolar and multipolar decrease and vice versa. Nevertheless, all three forms may occur at any level and in any size of piece within the limits of the experiments. This being the case, the only possible conclusion is that the observed frequency gradients of these forms are associated with and are expressions of the axial physiological gradient of the body.

In the very small pieces unipolar and bipolar partial forms consisting almost entirely of apical structures occur. These forms show that in hydra, as in various other animals (Child, 1915, 1924), the apical region of the individual arises independently of other parts.

*Tentacle formation.*—The data show that in both normal and depressed animals without buds (Tables VI and VII) the numbers of tentacles at any given time and the total numbers of tentacles found are greater in the distal and proximal than in the middle body regions. The distal pieces give rise to the greatest total numbers of tentacles, the proximal pieces are next, and the pieces from the middle regions show the smallest numbers. On the second day the distal pieces have more tentacles than the proximal pieces on the major axes, but later the number on the major axes of the proximal pieces approach, equal, or exceed those of the distal. However, the tentacle numbers during the period of observation in pieces

in good condition are greater than in depressed pieces—often twice as great.

These observations confirm those of Marshall (1892), Peebles (1897), Rand (1899), King (1903), and Koelitz (1910) for other species of hydra and serve to verify the data on susceptibility in hydra (Child and Hyman, 1919; Hinrichs, 1924). Apparently the distal region is one of high metabolic rate, since tentacle formation takes place here most rapidly after sectioning. The proximal region, which includes the budding zone, apparently is likewise one of somewhat higher rate than the middle region, but lower than the distal region, as is indicated by the slower rate of tentacle appearance for two days after cutting. The increase in rate of reconstitution in this proximal level affords further evidence that, though there was no visible indication of buds in these cut specimens, yet the budding zone, as indicated by its physiological activity, is ready to bud and is partially free from the dominance of the apical end.

The marked difference in the numbers of tentacles formed in a given time by pieces from animals in good condition compared with those in depression, together with the results obtained by Hyman (1927) on the susceptibility of depressed animals, indicates that the metabolic rate is low in such animals. The influence of this factor on reconstitution has already been discussed (pp. 201–3). Data on the rate of tentacle formation in pieces of young animals before they approach the budding period are necessary to complete the evidence along this line. On account of the difficulty in sectioning the small animals, these data have not yet been obtained.

*Bud formation and separation.*—Since the stalk of *P. oligactis* is apparently incapable of reconstitution, the budding zone represents the most proximal region which is capable of organization into a new individual, and, as the following experiments on budding indicate, a certain degree of physiological or physical isolation from apical dominance is necessary for the occurrence of budding. The data on tentacle formation indicate that some increase in activity of this region may occur before buds become visible.

The data show (Table VIII) that budding is followed by bud separation only if the distal one-half to two-thirds of the body region and the stalk are removed. If the stalk remains, the percentage of

bud separation is reduced to zero. This might seem on first sight to indicate that the budding zone, according to the theory of dominance and subordination, is controlled by both the more distal body levels and the stalk. Goetsch (1925), perhaps with this idea in mind, reports that removal of the stalk may release the budding zone from an inhibition and cause budding to take place. However, analysis of the data seems to show that the budding zone is dominated by the more apical body region, removal of which causes budding to take place. But if the stalk remains attached to the body, the bud migrates distally from its lateral position and, since the developing bud has now a higher rate of metabolism than the attached parent-piece, takes control of the proximal body level and stalk already formed.

One explanation for this behavior on the part of the bud under the circumstances above described is that the metabolic rate of the parent-piece with the stalk attached is not increased to any great extent, since the stalk attaches itself almost immediately after section. Thus any increase in metabolism due to movement is practically eliminated. If, on the other hand, the stalk is removed, there are present not only two areas of stimulation as a result of cutting but undoubtedly increased activity due to the unattached condition of the piece. Thus the metabolism of the parent-piece may be increased to such a marked extent that it is impossible for the apical region represented by the bud to take control of the piece, and consequently the bud separates. Rand (1899) and King (1903) noted that if a transverse section is made through a budding hydra so that a portion of a bud remains attached to the proximal region of the parent, the bud does not separate, but takes control of the parent-piece. Marshall (1892) reported a similar reaction between bud and proximal region in *Chlorhydra viridissima*. A similar situation occurs in *Planaria* when a new head region develops on a piece. The new reconstituted head region takes control of the structures already present and in part reorganizes, in part retains them.

The low percentage of budding in small pieces may result from the inhibition of budding by the parent-piece because of the proximity of the gradient arising at one or both of the cut ends. On the other hand, a lateral axis may arise which instead of migrating or

separating may absorb the parent-piece and thus reconstitute. In such instances it is impossible to determine whether or not this new axis represents a bud, and the piece is therefore classified as a unipolar form.

That conditions favoring budding are different in depressed from those in normal animals has been noted by many investigators who report buds on more distal levels which in many instances remain on the parent and form "colonies." The relation between budding and depression is brought out very clearly by Hyman (1927). Additional data on the relation of depression to budding are summarized in Table IV, column 8. In sectioning the same procedure was followed as in other cases, except that the stalk was removed from all pieces. It will be noticed that the body pieces cut into fourths show a relatively high percentage of budding, not only in the original budding zone, level 4, but in pieces from the entire proximal three fourths of the body. In sixths the proximal levels 5 and 6 show a marked increase in budding compared to the same levels of non-depressed specimens. This increase of budding and in extent of the budding zone in the pieces of depressed animals may be explained as being due to the much lower metabolic rate of these animals, which resulted in a decrease of dominance some time before section. Apparently the gradient is practically destroyed by section, except in the most distal levels, and with it the former relations of dominance and subordination. Under these conditions there has resulted a marked increase in budding.

It is interesting in connection with budding to consider briefly the theory which Goetsch (1919, 1920, 1925, etc.) has developed to account for the origin of buds and their relation to the parent-animal. According to Goetsch, a bud originates at a certain place where a large amount of food has accumulated, and will not separate from a parent-hydra from which the hydranth and stalk have been removed unless the parent has been fed previous to section, since there is not sufficient food to enable the bud to continue its development. For the same reason a bud will not separate from a very small body piece, but instead will either absorb or take control of it. Boecker (1921) has repeated the experiments of Goetsch on feeding parent-animals before sectioning and has been unable to verify this

conclusion. Moreover, it has been observed by many investigators on hydra that in starvation the buds separate from the parent-animal even more readily than they do under normal conditions. Likewise, the data presented above fail to support Goetsch's theory. As may be seen from Table I, buds have formed and separated from pieces comprising from one-half to only one-tenth of the total body region of the parent. Further, it is well known that hydras in a state of depression are unable to feed because of the loss of tentacles and closure of the mouth, and are consequently in a state of starvation. In spite of this buds arise and separate from parent-pieces varying in size from one-third to one-sixth of the body region (Table IV). Goetsch attempts to show further that the relation of dominance and subordination between bud and parent, as well as the phenomenon of regulation in pieces with double hypostomes, depends upon one or the other structure obtaining control of the available food supply. In view of the data already presented, it does not seem to be necessary to discuss this theory at greater length.

In general, the data presented indicate, first, that a polar gradient is present in hydra, and, second, that it is a factor in determining the form frequencies and the rate of reconstitution at different levels. The data indicate further that localization and extent of the budding zone and the formation and separation of buds are also expressions of gradient relations. This paper is concerned chiefly with presentation of data rather than with interpretations. Further experimental investigation is necessary in order to determine the physiological basis of the relation between form frequencies and body levels, for example, what particular factors determine that unipolar frequency increases from distal to proximal levels, etc. It is believed that work already in progress and other work planned will throw light on such questions.

## SUMMARY

1. In reconstitution pieces of the body region of *Pelmatohydra oligactis*, after the removal of the hypostome and stalk, give rise to different forms with a variable number of axes. Three classes of forms may be distinguished: unipolar, bipolar, and multipolar. The relative frequency of these forms in general depends upon the size

of the piece, the level of the body from which the piece was taken, and the general physiological condition of the animal.

2. Unipolar forms occur at all levels but increase in frequency, and bipolar and multipolar forms taken together decrease in frequency, from the distal to the proximal levels in pieces comprising one-third of the body or less. In pieces from distal levels ranging in size from one-third to one-sixth of the body region, the frequencies of unipolar forms vary directly and those of bipolar and multipolar forms inversely with the size of the piece. In general, the size of the piece has no effect on the total form frequencies of pieces including all body levels.

3. The occurrence of bipolar forms as the result of reconstitution has apparently not been previously noted in hydra, but in this species bipolar forms ("axial heteromorphosis"), which may conceivably involve either a reversal of polarity at the proximal or the origin of two new polarities, occur frequently, particularly at the more distal body levels.

4. Pieces of all sizes and from all levels of depressed *P. oligactis* give a high unipolar frequency irrespective of the size of the piece. It is suggested that partial obliteration of the gradient in consequence of the physiological condition of the animals, decreased motor activity, and the action of a contact differential may all be concerned in determining this high unipolar frequency.

5. Data on differential susceptibility indicate that the determination of axes in a piece and hence of its form takes place under the usual conditions within twenty-four hours after section. The various axes of a piece are determined by the loci of the regions of high metabolic rate.

6. The presence of a primary apico-basal gradient and a secondary increase in metabolic rate in the budding zone is indicated by the rate of tentacle formation. Daily tentacle counts during the first five days following section show that in the earlier stages of reconstitution distal pieces possess the largest numbers of tentacles, proximal pieces smaller numbers, and pieces from the middle region the smallest numbers. The total numbers of tentacles formed by distal and proximal pieces within five days following section exceed those of pieces from the middle body region. These data were ob-

tained from non-budding animals both in good condition and in depression.

7. The rate of tentacle formation on pieces from depressed animals is approximately one-half that of the pieces from animals in good condition.

8. Budding can be experimentally induced in the budding zone by removal of more distal body regions, but whether the bud remains and takes control of the parent-piece or separates depends on the presence or absence of the parent-stalk. No evidence of a dominance based on control of food supply has been observed.

9. The stalk fails to regenerate in the absence of body tissue.

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# THE EFFECT OF A HORIZONTAL TEMPERATURE GRADIENT ON THE DEVELOPMENT OF THE EGG OF THE URODELE, TRITURUS TOROSUS

(Sixteen figures)

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## INTRODUCTION

MANY studies have been made to determine the effect of variation in temperature on the development of eggs; but, so far as I am aware, until recently there has been little done to find out what the effect of a gradient of temperature might be. In December, 1926, Julian Huxley reported an experiment in which he applied a vertical temperature gradient to cleaving frog's eggs. He compressed newly fertilized eggs lightly between an upper and a lower chamber, in one of which there flowed warm water, and in the other, cold water. He maintained the temperature difference for from ten to twenty hours. As a result he observed a modification in the size of cleavage cells and in the size of the head of the embryo that developed. If the temperature was descending, "adjuvant" as he styles it, the animal cells were abnormally small in comparison with the vegetal cells, and the head that developed larger than usual. If, on the other hand, the gradient was ascending, "antagonistic" as he says, the opposite results were noted. In a more recent study, Huxley and Tazelaar (1927) have applied a horizontal temperature gradient to the incubating hen's egg.

In this paper I wish to report experiments performed during the spring of this year (1927) at Claremont, California, in which, by a method entirely different from that used by Huxley, I applied a horizontal temperature gradient to the cleaving eggs of the Pacific Coast newt, *Triturus torosus* (Rathke). Preliminary experiments were begun on the problem at Berkeley in 1924.

The method suggested itself to me several years ago as a means by which the hypothesis of metabolic gradients might be tested. It

is held by Child (1924, 1925), Hyman (1921, 1926, 1927), Bellamy (1919), and others that the primary determining features in the differentiation of eggs are physiological gradients; that is to say, gradations in the rate of the fundamental physico-chemical processes of living substance. If this view be accepted, then we should expect to find that any method which controls the rate of metabolic activity would modify the differentiation of the egg. Now, what could be better fitted to control physico-chemical processes than differential warming? Surely a gradation in temperature should effect a gradation in the rate of metabolic change.

The preliminary studies and the analysis of the present results were done in the laboratories of the department of zoölogy, University of California, Berkeley, California. I am especially grateful to Dr. J. Frank Daniel, of that department, for his unfailing interest in my work, and for helpful suggestion. The experiments themselves were performed in the zoölogy laboratory of Pomona College, Claremont, California.

## MATERIAL AND METHOD

The apparatus employed consisted of a warm and a cold water bath, and of paired aluminum plates, hereafter called "thermodes" (see Fig. 1). One thermode of each pair was immersed in the warm bath at one end and conducted heat to one side of a row of amphibian eggs at the other; while the other thermode of the pair was immersed in the cold bath at one end and conducted heat away from the opposite surface of the same row of eggs at the other end. Thus a temperature gradient was established and maintained across the eggs. The experiment was first attempted using water as a medium with which to surround the eggs; but the results obtained were not satisfactory, due probably to convection currents in the water. Moreover, the orientation of the eggs could not be determined with certainty. To overcome these difficulties I have this season employed a thin agar jelly as a matrix about the eggs, and with highly satisfactory results.

The water baths were wooden tanks approximately $13 \times 13 \times 46$ cm. ($5 \times 5 \times 18$ in.) in inside dimensions. The temperature of the warm bath was maintained by two electric-light bulbs and a thermo-

stat (not shown in the figure); that of the cold bath by emptying into it a continuous stream of water chilled by flowing through a copper coil immersed in ice water. It was found that within limits the rate of flow had little influence on the temperature of the bath; but that the temperature was controlled principally by the length of copper coil immersed in the ice water.

The thermodes were plates of 3-mm. (actually $\frac{1}{8}$ in.) aluminum, approximately 6×15 cm. ($2\frac{1}{2}$×6 in.) in size, and bent as indicated

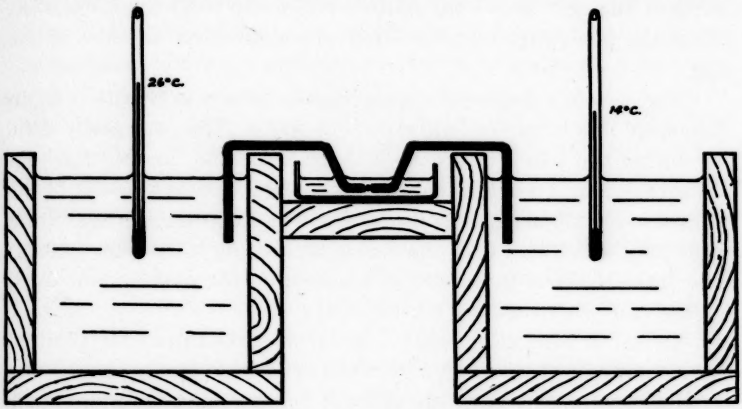


FIG. 1.—Cross-section of the temperature-gradient apparatus. There is shown a warm water bath (left), a cold water bath (right), and a pair of aluminum plates or "thermodes" (heavy black lines). The eggs were placed in a petri dish between the thermodes and surrounded by thin agar jelly. ×$\frac{1}{4}$.

in Figure 1. Four pairs of thermodes were used between the two water baths.

The eggs were arranged in petri dishes on a shelf between the water baths. It was possible to accommodate fifty-six eggs at one time.

The agar jelly used as a medium about the eggs was a one-fourth of 1 per cent solution of a commercial product manufactured in Glendale, California. It was found that when heated to boiling the agar dissolved, but that then the solution might be cooled to well within the limits of toleration of the eggs before it finally set to a jelly.

An experiment was set up as follows: The petri dishes were first placed in position on the shelf between the water baths, and a very little agar solution, which had been heated to fluidity, poured into each. While the agar was setting to a thin jelly the thermodes were put in place, their proximal ends touching each other. They were then immediately drawn apart to the proper distance to accommodate a row of eggs. By this means a shallow agar trough was formed between the thermodes, in which to place the eggs, and by which to support the eggs above the bottom and away from the thermodes. Thus the possibility of respiration was assured on all sides of the egg.

The eggs of a single jelly mass, usually fifteen to twenty in number, were now removed into a dish of water. This was easily done by slicing the jelly away with a sharp knife and floating the eggs off into water. To determine the exact stage in development of the eggs removed, one egg was dropped into a little viscous agar (thin agar jelly which had been emulsified by forcing it through a cloth) and rotated about by means of a hair from the eyebrow mounted on the end of a dissecting needle.

Twelve or fifteen eggs of the cluster were then placed in position between a pair of thermodes by means of a wide-mouthed medicine dropper. Ordinarily each egg of itself rotated until its animal pole was upward, but occasionally a light touch with the hair needle was necessary to bring an egg into position. There were no landmarks observed on the surface of the eggs of *Triturus* by which any definite horizontal orientation could be effected.

As soon as a row of eggs was in place between a pair of thermodes, a few drops of agar solution which had been cooled to about 28° C. was carefully poured upon them. A moment later, when this had jelled, more agar was added, and so on until the eggs and the ends of the thermodes were covered 3 or 4 mm. deep with thin agar jelly.

To prevent evaporation, the shelf between the water baths was entirely inclosed by wood and glass; yet, in spite of this precaution, evaporation occurred, and it was found necessary to add a little water from time to time.

In most of the experiments hereafter described the eggs were sub-

jected to a temperature gradient of 12° between water baths for a period of about forty hours. The heating and the cooling of the water baths were then discontinued, but the thermodes and the eggs were not disturbed for some days longer, since it was found impossible to remove the thermodes without causing rotation and dislocation of the eggs. As soon, however, as the neural plate was clearly visible and the neural folds had begun to rise (see Fig. 3*B*, p. 238), a drawing was made of the eggs as they lay *in situ*. Each egg was then carefully removed, reoriented in viscous agar in the position which it had occupied in the machine, and again carefully drawn; the positions of the hot and cold poles were indicated, and drawings from other aspects made. Most of the eggs were now preserved at once, but some were permitted to develop to a later stage before preservation.

A study of the physical characteristics of the heat-conducting system of the apparatus has shown that the temperature descends much more rapidly in the region of agar jelly between the thermodes than it does in the thermodes. (The thermal conducting coefficient of aluminum is nearly three hundred times as great as that of pure water.) In effect the thermodes brought the temperature of the water baths to the opposite surfaces of the eggs with only a small decrement. The averages of a series of actual measurements taken at different points in the system are given in Figure 2. The abscissae indicate linear distance in the direction of heat flow. *A* and *F* are the water baths; *B* and *E* are points on the thermodes just after they have emerged from the water baths; *C* and *D* are points near their proximal ends. It will be seen that the temperature falls most rapidly between *C* and *D*, the region of lowest heat conductivity. Of the twelve isotherms which cross the system, seven pass through the agar jelly.

The egg occupied about three-fourths of the interval between the thermodes. Although the heat-conducting coefficient of the egg protoplasm has not been determined, yet it is probably of the same order as that of agar jelly, since both are aqueous and highly viscous. On this assumption each egg would be subjected to a difference of temperature of approximately 5° between opposing surfaces.

CONTROLS

The experiments were controlled in two ways. A study was made of the effect of agar jelly alone on development, and also of the effect of agar jelly and aluminum. Under both conditions numerous eggs developed normally. There is no question in the mind of the

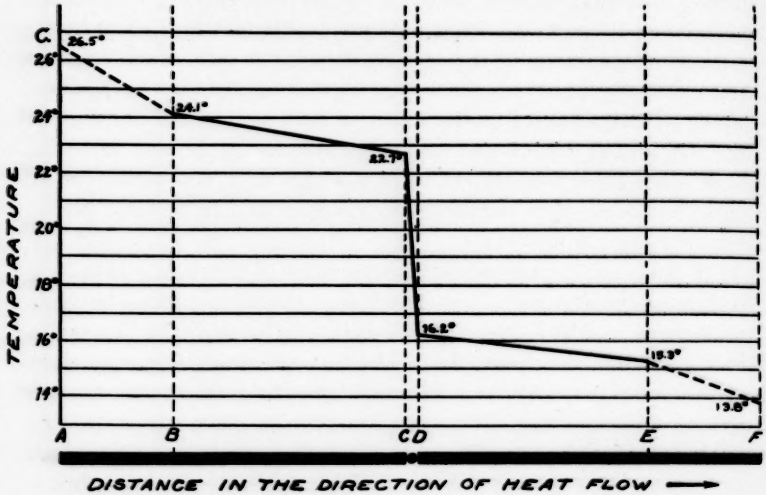


FIG. 2.—Physical characteristics of the heat-conducting system. Abscissae are distances in the direction of heat flow. Ordinates are temperatures. *A* and *F* indicate the temperatures of the water baths; *B* and *E*, the temperatures of the thermodes immediately after emerging from the water baths; *C* and *D*, the temperatures of the thermodes at points near their proximal ends. The distance *CD* in the figure is exaggerated twice. It will be noted that the fall in temperature in the agar jelly (*CD*) is much more rapid than in the thermodes (*BC* and *DE*).

author but that in themselves agar and aluminum were adapted to the present experimentation. No greater loss of life occurred among the agar-aluminum controls than among the agar controls; and, furthermore, when the agar jelly was thin enough, shallow, and when water was added to make up for evaporation, the viability of eggs in the jelly was practically equal to that in water.

There were, however, cases of retarded development and death among the controls, as there were also among the eggs which devel-

oped in pure water. The probable causes of such losses will now occupy our attention briefly.

*a*) When the eggs were removed from the jelly mass and placed in water, it was not uncommon for several of the eggs to die. This was probably a result of injury sustained from the operation, or due to sudden change in temperature, surface tension, or osmotic conditions.

*b*) If the water of the agar jelly became fouled as the result of the death and disintegration of an egg, the other eggs in the solution became retarded in their development. This was especially true of the early stages, before the ectoderm had become resistant to surrounding conditions. In one respect agar jelly showed an advantage over water as a medium; namely, in that it limited the spread of the products of decomposition to the immediate vicinity of the disintegrating egg.

*c*) An insufficient supply of oxygen for respiratory needs was apparently a frequent cause of the failure of eggs to live and to develop. In nature the eggs of *Triturus* are laid in flowing mountain streams or in cool, shaded ponds and lakes. Under such conditions they do not ordinarily suffer a lack of oxygen. But I have repeatedly observed that when egg masses are brought into the warm laboratory and covered deeply with water they cease to do well. Under laboratory conditions I have had better success with eggs which have been removed from their jelly and placed in shallow water. There is apparently an increased demand for oxygen in the warmer environment which can best be met by placing the eggs in a shallow dish with but little water.

Again, I have observed that experimentally weakened eggs, especially those which have lost their vitelline membranes, do not do well if placed in contact with the bottom of a glass dish. They not infrequently disintegrate on the under side, and may even stick to the substratum. I have found it helpful to place such eggs in dishes the bottoms of which are covered by a thin layer of agar jelly. Spemann used sheets of gelatin for the purpose, but had to change the gelatin quite often in order to avoid the development of micro-organisms. The jelly supports the eggs above the bottom of the dish and

permits respiration on the under side, thus simulating the condition of the eggs in their natural jelly.

The cooling agar solution which was poured over the eggs subjected them for a brief interval to an unduly warm medium of low oxygen content. There seems, however, to have been little loss of life from this cause (unless the agar were deep), probably because the eggs retained sufficient oxygen in their cytoplasm to supply their needs until the oxygen content of the jelly and a lower temperature were restored.

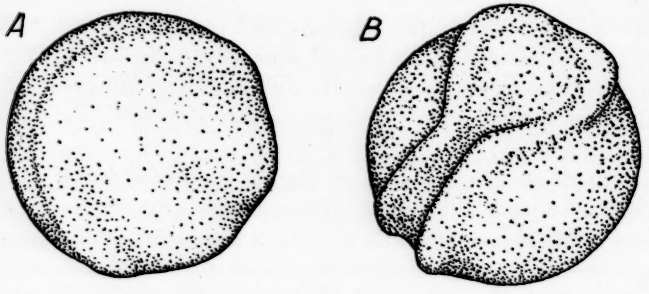


FIG. 3.—Control eggs viewed directly from above. When the brain area first appeared it was almost always upward (*A*); but with the rising of the neural folds a gradual rotation of the egg within the vitelline membrane occurred (*B*). Usually the rotation was as shown, but sometimes it was forward or backward. ×20.

Especial attention, in the case of the agar controls, was given to the matter of the rotation of the egg within the vitelline membrane. It was found that the neural plate, at the time of its first appearance, had a uniform position with respect to gravity (see Fig. 3*A*). The forebrain area of the neural plate was commonly on top. But, with the appearance of the neutral folds a slow rotation of the egg almost invariably began, usually forward and to the side (Fig. 3*B*) but sometimes forward and even backward. The rotation, however, was never so great as to bring the blastopore to the upper side of the egg.

I have not recorded cases of asymmetric neurulae from among the agar controls. Asymmetry does indeed appear later in development, when the embryo has begun to elongate; but its cause then is a mechanical one. Normally an elongating embryo is able by its

bending movements to burst the vitelline sac in which it first develops and straighten itself out; but in the agar controls the jelly supports the sac membrane and prevents its rupture. As a consequence the embryo is forced to take the shape of a horseshoe with the tip of the head close to the tip of the tail. This deformed shape is retained even when the embryo is finally released from its confinement.

Eggs in glassware with water as a medium often develop asymmetry of another sort. When the neural folds appear they revolve to the side, with the result that the two sides become subjected to unlike environmental conditions. Under such circumstances the embryos not infrequently show curved axes.

The agar controls usually died about the time the gills became functional. When older larvae are imbedded in agar jelly it is the gills which first begin to disintegrate. This is probably not due to any toxicity of the agar jelly, but to the fact that it prevents the normal swimming and respiratory movements of the larva.

## EXPERIMENTS

Two hundred and fifteen eggs in all were subjected to a temperature differential during the present experimentation. Fifty-five of these were put in the gradient apparatus while yet in early cleavage (8–96 cell stages); 68 were in the mid-blastula stage; 26 were late blastulae; 14 were in earliest gastrulation; 14 were in mid-gastrulation (blastopores semicircular); and 14 were in late gastrulation (earliest yolk plug). This paper will deal with the results obtained from the first two sets of eggs. The remaining experiments will be reported at a later time.

Of the 123 eggs now under consideration, 39 either died while yet in the thermal gradient apparatus, or else were so weakened that they burst during the processes of removal and study. The causes of this loss will be discussed later under the topic of the viability of the experimental eggs. The 84 eggs which lived form the basis of this report. Each of them has been assigned an individual number.

The 84 eggs may be classified according to their experimental handling as follows:

*Group I.*—Eggs in early cleavage at the start of the experiment; temperature gradient, 26°–14° C.; duration of gradient, 40 hours—30 eggs (eggs numbered 16–22, 43–56, and 57–65).

*Group II.*—Eggs in early cleavage at the start of the experiment; temperature gradient, 26°–14° C.; duration of gradient, 58 hours—9 eggs (eggs numbered 1–9).

*Group III.*—Eggs in mid-blastula stages at start of experiment; temperature gradient, 21°–14° C.; duration of gradient, 39 hours—11 eggs (eggs numbered 88–98).

*Group IV.*—Eggs in mid-blastula stages at start of experiment; temperature gradient, 26°–14° C.; duration of gradient, 40 hours—34 eggs (eggs numbered 31–42, 66–87).

Since careful study has shown similar types of abnormalities in each of the foregoing cases, no attempt will be made to keep the several groups separate in the description of results which follows.

Nineteen of the eggs (eggs 16–22 and 31–42) were disturbed just before their removal, and notes as to their orientation with respect to the thermal gradient could not be made. The data for the remaining 65 eggs are complete.

## RESULTS

*Introductory statement.*—The results of these experiments, in which a horizontal temperature gradient was applied to blastulae of the newt, will be described under three heads: (1) the general viability of the eggs; (2) the rotation of the eggs during the experiment; and (3) the types of abnormalities which appeared. Under each of the heads we shall consider, first, the phenomenon in itself, and, second, the phenomenon in its relation to the direction of the thermal gradient across the egg.

The problem of the phenomenon in itself has offered no difficulty other than that of careful and controlled observation. The problem of the phenomenon in its relation to the direction of the thermal gradient, on the other hand, has presented difficulty. In the first place, the eggs were not oriented in a definite horizontal direction when subjected to the thermal gradient, there being no visible surface features observed by which such orientation could be effected. Observations as to orientation were made at the neurula stage, and have been given retrospective value. The visible orientation of the

neurula has been taken as an index of the invisible orientation of the egg at the beginning of the experiment.

The last statement is in need of modification. In the experimental eggs the neural plate seldom occupied exactly that position with respect to gravity which it occupied in the controls (see Fig. 3*A*); but was almost invariably displaced to a greater or less extent, either laterally, or backward, or, less often, forward. Two explanations of the displacement are possible: (1) The neural plate may have differentiated in an abnormal position; or (2) the egg as a whole may have rotated within its vitelline membrane. There is a small amount of evidence in favor of the first possibility: The neural plates were not always of the same size, and sometimes they showed curvature; but that they were ever actually shifted in position to another portion of the egg's surface is not to be concluded on the basis of the evidence at hand. There is good reason to believe that the second explanation is the correct one. A slow rotation of the egg almost invariably occurred in the controls while the neural folds were rising; moreover, it was observed among the experimentals. Fortunately for the experiments, the rotation was seldom great in amount and did not vitiate the results.

In view of the rotation, however, it has been thought proper in interpreting the orientation of an egg to determine by inspection as nearly as possible the axis of the egg's rotation (presumably horizontal), and then to consider the original position of the egg as that which it would assume were it rotated back until its dorsal side is on top. This correction has been applied in the tables and in the schematic figures which follow. Except in four marginal cases the correction does not alter the interpretation as to which side of the egg was warmed and which was cooled.

*Tabulation of the experimental eggs.*—In Table I there is indicated for each of the experimental eggs: (*a*) the position on the egg of the warmed meridian, (*b*) the direction of the rotation of the egg, and (*c*) the more notable types of abnormalities which the egg showed. The position of the warmed meridian is given as D, dorsal; DL, dorsal left; L, left; VL, ventral left; V, ventral; VR, ventral right; R, right; or DR, dorsal right. The direction of rotation is stated as

TABLE I

| Egg | Warmed Meridian | Direction Rotation | Abnormalities |
|---|---|---|---|
| 1..... | ? | ? | Incomplete blastopore; NP indicated by pigment only |
| 2..... | ? | ? | Incomplete blastopore; NP absent; secondary (?) puckering on L |
| 3..... | ? | ? | Gastrulation barely begun; NP absent |
| 4..... | V | L | Neural axis concave toward R |
| 5..... | DR | B | NF larger on R |
| 6..... | DR | B | Large yolk plug; NF slightly larger on R |
| 7..... | ? | ? | Large yolk plug; NP absent |
| 8..... | DL | B | NF larger on L; blastopore toward R |
| 9..... | DL | B | Small yolk plug; NP small |
| 16..... | ? | ? | Normal |
| 17..... | ? | ? | NF larger on R |
| 18..... | ? | ? | Neurulation retarded but apparently normal |
| 19..... | ? | ? | Incomplete blastopore; primary puckering |
| 20..... | ? | ? | NF larger on R; secondary puckering on R (Figs. 11*A* and 12) |
| *26..... | ? | ? | Accessory brain area on R (Figs. 13*C* and 14) |
| *30..... | ? | ? | NF larger and darker on R; secondary puckering on R (Fig. 11*B*) |
| 33..... | ? | ? | Accessory NP on R (Figs. 13*F* and 15) |
| 35..... | ? | ? | Small yolk plug; neural axis concave toward R; aberrant NF on L |
| 37..... | ? | ? | Secondary puckering and aberrant NF on R (Fig. 13*A*) |
| 38..... | ? | ? | Large and in part aberrant NF on L; neural axis concave toward R |
| 39..... | ? | ? | Accessory brain area on L (Fig. 13*B*) |
| 40..... | ? | ? | NF larger and darker on R; neural axis concave to L |
| 41..... | ? | ? | NF larger and darker on R |
| 42..... | ? | ? | NF larger and darker on L; neural axis concave to R |
| 43..... | D | B | NP short; anterior NF large (Fig. 6*A*) |
| 44..... | R | B | Slight secondary puckering on R |
| 45..... | DR | L | NF larger on R; secondary puckering or accessory NP on R |
| 46..... | DL | R | NF larger on R; accessory NP on L |
| 47..... | ? | ? | Large yolk plug; abnormal NP with small brain area; secondary puckering (?) (Fig. 6*B*) |
| 48..... | ? | ? | Small yolk plug; NP small; aberrant NF on L |
| 49..... | DR | R | Small yolk plug; NP short; NF larger on R |
| 50..... | DL | L | NP short; NF larger on L; blastopore toward R |
| 51..... | D | B | Large yolk plug; abnormal NP with small brain area |
| 52..... | DR | L | Small yolk plug; NF larger on R; accessory NP on R (Fig. 13*E*) |
| 53..... | DL | R | NF larger and abnormal on L |
| 54..... | DR | R | NF abnormal on L (Fig. 13*D*) |
| 55..... | DR | R | Large yolk plug; NF abnormal on L |
| 56..... | R | R | NP short; anterior NF large |
| 57..... | VR | R | NF slightly larger on R |
| 58..... | D | B | NF larger on L |
| 59..... | ? | ? | Incomplete blastopore; primary puckering |
| 60..... | ? | ? | Incomplete blastopore; primary puckering resembling NF |
| 61..... | V | F | NF somewhat disorganized on R |
| 62..... | ? | ? | Large yolk plug; primary puckering (Fig. 5*A*) |
| 63..... | V | B | NF larger posteriorly on R |
| 64..... | ? | ? | Small yolk plug; indication of a horizontal NF |
| 65..... | ? | ? | Large yolk plug; NP indicated by pigment (?) |

TABLE I—*Continued*

| Egg | Warmed Meridian | Direction Rotation | Abnormalities |
|---|---|---|---|
| 67..... | VR | F | Incomplete blastopore; fairly normal NP |
| 68..... | L | L | NF larger on L |
| 69..... | DL | R | NF larger on L (Figs. 7*B* and 8) |
| 70..... | L | L | NP somewhat short; NF larger on L (Fig. 9) |
| 71..... | VR | L | NF larger on R (Fig. 7*C*) |
| 72..... | L | O | NF larger on R (except forebrain area which is larger on L) |
| 73..... | VL | R | NF larger on L |
| 74..... | VL | O | NF larger on L |
| 75..... | D | R | NF larger on R |
| 76..... | DL | L | NF larger on L (Fig. 7*D*) |
| 77..... | VL | L | NF larger on L |
| 78..... | DL | B | Incomplete blastopore; NP retarded |
| 79..... | DL | O | Large yolk plug; slight horizontal NF |
| 80..... | DL | R | Incomplete blastopore; NF larger on L |
| 81..... | R | O | Large yolk plug; NF larger on R |
| 82..... | L | O | Large yolk plug; primary puckering resembling NF (Fig. 3*B*) |
| 83..... | R | R | Large yolk plug; NF larger on R |
| 84..... | VL | B | Large yolk plug; very short NP; large anterior NF |
| 85..... | DR | B | Abnormal NP |
| 86..... | D | O | Large yolk plug; horizontal NF (Fig. 5*C*) |
| 87..... | VL | R | NF aberrant on L |
| 88..... | DL | B | NP short; NF larger on L |
| 89..... | VR | B | NF larger on R (Fig. 7*A*) |
| 90..... | VL | R | NF larger on L |
| 91..... | DL | R | NF larger anteriorly on L; neural axis concave toward R |
| 92..... | V | O | Fairly normal |
| 93..... | ? | ? | Large yolk plug; NP indicated by pigmentation |
| 94..... | VR | O | Small yolk plug |
| 95..... | D | O | Large yolk plug; NF larger on L |
| 96..... | DR | O | Small yolk plug; NP indicated by pigmentation |
| 97..... | VR | O | NF larger on R |
| 98..... | VL | L | NF larger on L |

* These two eggs were subjected to the gradient when in late blastula stage, but are referred to later in this paper.

F, forward (i.e., so as to bring the neural plate to the upper half of the egg); B, backward (so that only the tip of the brain area appears above the equator); R, to the egg's right; and L, to the egg's left. In listing the abnormalities the abbreviations NP, neural plate; NF, neural fold or folds; R, right; and L, left have been used. The different descriptive terms employed will be explained later.

*Viability.*—The death-rate among the experimental eggs was high. Only 84 out of 123 survived; and of these 84 a considerable number were backward in their development and would soon have died. In some cases death was clearly due to injury sustained by an egg during handling, or to an egg becoming flattened against a

thermode when it was placed in the apparatus. Unfortunately, eggs that died could not be removed without disturbing other eggs in the vicinity and rendering observations as to their orientation not trustworthy. The disintegration of an egg retarded the development of other eggs in its immediate vicinity; and thus the death of 1 egg led to the loss of 2 or 3 others before the experiment could be ended. In view of these facts, the loss of 39 eggs out of 123 is perhaps not surprising. There is no reason to conclude that either the agar jelly or the aluminum of the thermodes were the causes of death. In fact, the agar jelly tended to minimize the loss rather than otherwise; for if in a dish of eggs with water as a medium, an egg should die and not be removed, it was usually only a short time until the whole solution was contaminated.

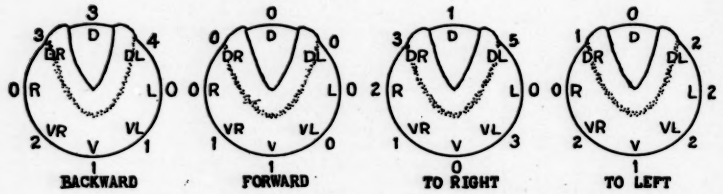


FIG. 4.—Diagram to indicate the positions of the warmed meridians of the eggs which rotated (*a*) backward, (*b*) forward, (*c*) to the egg's right, and (*d*) to the egg's left. The numerals indicate the instances in which a given meridian was warmed.

The thermal gradient may occasionally have so influenced the processes of development as to render an egg non-viable; but this is not proved. A tabulation of the orientation of the eggs, which survived with respect to the direction of the thermal gradient, has revealed no correlation of consequence. A very few more eggs lived on which the dorsal side had been warmed than on which the ventral side had been warmed; but the figures do not warrant the conclusion that warming the ventral side is inimical to the egg's chances of development.

*Rotation of eggs.*—The rotation of an egg within its vitelline membrane has already been described. It remains now to discuss the relation of the direction of this rotation to the direction of the thermal gradient across the egg. The matter is summarized in the schematic Figure 4. The numbers indicate the positions of the warmed

meridians on the eggs which rotated (*a*) backward, (*b*) forward, (*c*) to the egg's right, and (*d*) to the egg's left. The numbers are small; yet some correlation may perhaps be indicated. Rotation backward, so that only the anterior tip of the neural plate appeared above the equator, appears to be correlated to some extent with warming the dorsal side of the blastula. Of the 14 examples of such rotation, 10 were warmed on the dorsal side, 4 on the ventral side. Forward rotation occurred in clear-cut fashion in only 2 instances. In both of these the ventral side was warmed. Whether or not a correlation exists between rotation to the right or left and the direction of the thermal gradient cannot be determined from the few cases observed. It is not impossible, on the basis of the small amount of evidence, that warming the dorsal side favors rotation to the right; although such a relation is hardly to be expected. There is undoubtedly a large element of chance in the direction of an egg's rotation; much depending on any slight tilt which the egg axis may have had at the beginning of the experiment.

*Types of abnormalities.*—The twenty or more types of abnormalities which have been listed in the tabulation of the experimental eggs may be classified under five general heads. It will be understood that in all cases the eggs were old enough at the time of tabulation to be neurulas with closed blastopores. The numbers in parenthesis in Table II indicate the occurrences of each type of abnormality. Little if any experimental significance is assigned to the abnormalities of Groups A, B, and C, on the basis of the evidence at hand. They are included mainly for the sake of completeness. The chief interest centers in the better assured results of Groups D and E.

A. *Retarded gastrulation.*—This abnormality was observed in 34 of the eggs which have been tabulated; and was present in aggravated form in most of the eggs which were lost before and during removal and study. The condition was observed among the controls; and is not to be assigned experimental significance. It was probably a result of inimical environmental factors, such as injury sustained when handled or the toxic effect of disintegrating eggs in the vicinity. Backwardness in gastrulating was commonly associated with certain defects of the neural structures; which defects will now be described.

B. *Retarded neurulation.*—The term has been used to describe any condition in which the development of the neural plate had not progressed far enough to reveal recognizable abnormality. It was invariably associated with backwardness in gastrulation, and no experimental significance is to be attached to it.

TABLE II

A. Retarded gastrulation
  Gastrulation barely begun (3)
  Incomplete blastopore (8)
  Large yolk plug (15)
  Small yolk plug (8)
B. Retarded neurulation (2)
  Neural plate absent (3)
  Neural plate indicated by pigment only (4)
C. Defective neural structures
  Primary puckering (3)
  Primary puckering resembling neural folds (2)
  Horizontal neural fold (3)
  Neural plate small or short (9)
  Large anterior neural fold (3)
  Abnormal neural plate (3)
D. Asymmetric neural structures
  Neural fold larger on right or left (40)
  Neural fold darker on right or left (4)
  Neural axis concave toward right or left (6)
  Blastopore toward right or left (2)
E. Accessory neural structures
  Secondary puckering on right or left (5)
  Aberrant neural fold on right or left (7)
  Abnormal neural fold on right or left (3)
  Accessory brain area on right or left (2)
  Accessory neural plate on right or left (3)

C. *Defective neural structures.*—Under this head have been grouped all those abnormalities of the dorsal surface which cannot be classified as accessory formations nor as asymmetries of development. It is hence a miscellaneous designation.

In the more extreme cases of the sort there were areas of irregular knobs and ridges; a condition which I have termed "puckering" (see Figs. 5*A* and 5*B*). I do not here include the wrinkling which occurs when the blastocoele collapses. When puckering takes

the place of a neural plate, I have called the condition "primary puckering." When, on the other hand, it occurs in addition to the neural structures, I have designated it "secondary puckering."

Primary puckering, at any rate in its more typical occurrences, is quite clearly related to neural folds. It is possible to choose, from among the experimental eggs, a series beginning with those which show extreme puckering, and ending with eggs which possess normal neural plates. Eggs 62, 82, and 86 may be taken as members of such a series (see Fig. 5). Each of them failed to complete gastrulation. Egg 62 (Fig. 5*A*) shows irregular ridges which, by reason of their position and pigmentation (pigmentation is not indicated in the

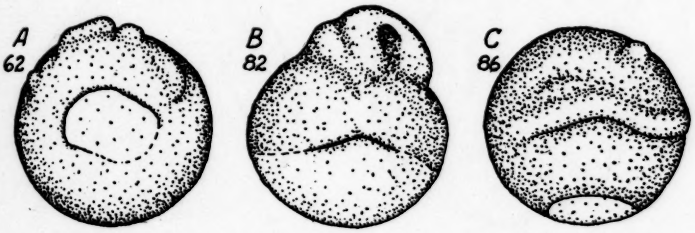


FIG. 5.—Defective neural structures. *A* (egg 62) shows "primary puckering"; *C* (egg 86) shows a horizontal neural fold; *B* (egg 82) represents an intermediate condition. ×15.

drawing), suggest a relationship to neural folds. In egg 82 (Fig. 5*B*) this homology between puckering and neural folds is even more apparent. Egg 86 (Fig. 5*C*) possesses what is very clearly a neural fold, but one which is transverse and equatorial in position (horizontal neural fold).

Puckering occurred in a few cases among the controls, but not often in so typical a fashion as that just described. Our interest in it is, therefore, not the experimental significance which may be assigned to it; but rather the probable theoretic significance which it bears to certain secondary puckerings yet to be referred to.

Nine eggs are listed as showing neural plates which were small or short (see Fig. 6*A*). A number of other eggs might have been included in the list had the criterion chosen been less strict. In normal eggs the neural plate extends over somewhat more than one-half of

a circumference, and cannot, therefore, be seen in its entirety from any one point of view. But in those eggs described as having small or short neural plates the entire neural axis was visible from a single aspect. The number of instances was too few to justify conclusions; yet there may be noted some correlation between eggs with short neural axes and rotation backward; and again between these two features and the previous warming of the egg on the dorsal side. In fact, 5 eggs of this description were warmed on the dorsal side, as against 1 which was warmed ventrally, and 2 which were warmed laterally. Three of the eggs which showed a short or small neural

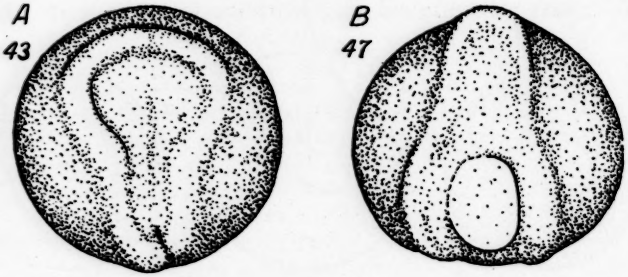


FIG. 6.—Defective neural structures. *A* (egg 43) shows a short neural plate; *B* (egg 47) shows an abnormal neural plate with small brain area, but with large folds lateral to the blastopore. ×20.

plate had very large anterior neural folds. The condition of 2 of the 3 eggs which have been tabulated as having abnormal neural plates is illustrated by Figure 6*B* (egg 47). There is shown a large yolk plug and a neural plate; but the anterior portion of the neural plate is small and not organized as a brain area, while its posterior portion is enlarged and extends as large folds on each side of the blastopore. The interpretation of this abnormality is not clear.

D. *Asymmetric neural structures.*—By far the most frequent abnormality observed among the experimental eggs was that of asymmetry in the size and pigmentation of the lateral neural folds (see Fig. 7). Egg 89 (Fig. 7*A*), drawn at the earliest neural fold stage, shows a greater development of the neural fold on the right side. In fact, the left fold is not as yet visible on the surface posteriorly. The plate is otherwise quite normal. Egg 69 (Fig. 7*B*), drawn in

the middle neural fold stage, reveals a left fold which is much the larger and which has been the more active in rising up. Two cross-sections of this egg (69) are shown in Figure 8, taken at the levels indicated by the cross-lines in Figure 7*B*. It will be noted that the

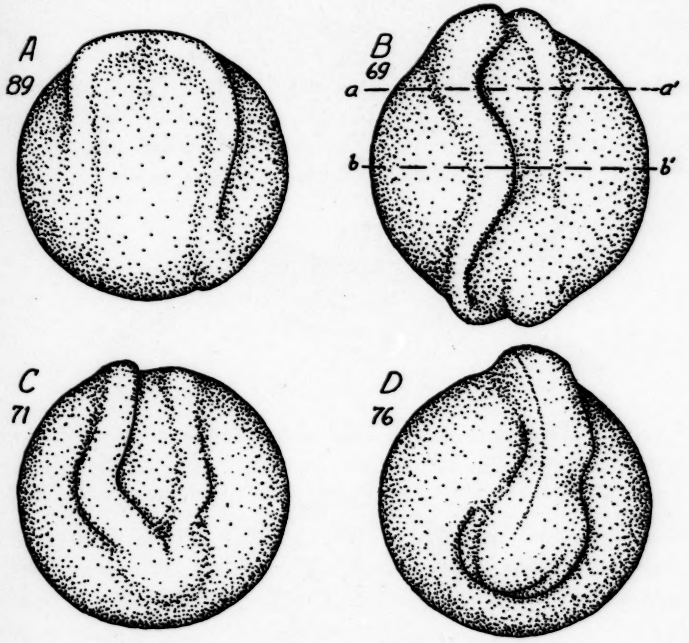


FIG. 7.—Asymmetric neural folds. *A* (egg 89) and *B* (egg 69) are dorsal views. *C* (egg 71) is an anterior view. *D* (egg 76) is an anterior view of a later stage than *C*. The deflection of the head to the side in *C* and *D* will be noted. In each instance shown the larger neural fold developed on the previously warmed side. ×20.

larger neural fold, failing to meet its fellow, has bent more sharply on itself than is normal, and is pushing across the midline. Not only is this neural fold further along in its development, but the ectoderm and neural crest of this side are also precocious in their development. Figure 7*C* is an anterior view of egg 71. The right neural fold is in this case the larger, and a slight deflection of the forebrain area to the left will also be observed. This deflection became even more

pronounced as the embryo developed. There was also asymmetry in the size of the optic lobes. A later stage in development, in which the neural folds have fused, is shown in Figure 7*D* (egg 76). The

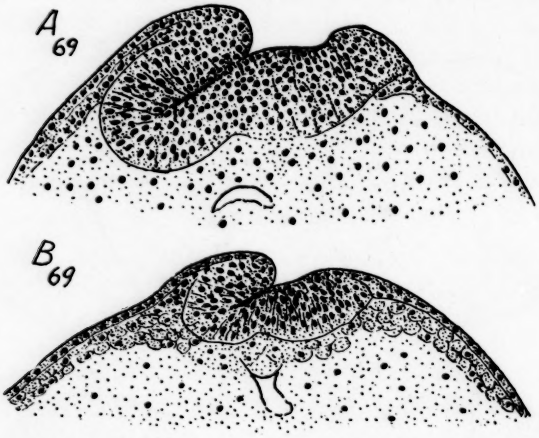


FIG. 8.—Asymmetric neural folds. Cross-sections of egg 69 at the levels indicated by straight lines in Fig. 7*B*. ×50.

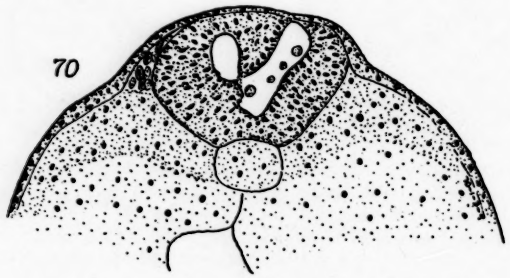


FIG. 9.—Asymmetric neural folds. Cross-section of egg 70 at an early neural tube stage. The large neural fold (left) has failed to meet its fellow and has fused with the floor of the neural tube. Later the smaller neural fold (right) has fused with the side of the larger; thus two neurocoeles have formed. ×50.

line of suture between the folds is still visible, and reveals the left fold to be the larger. The larger fold was also more darkly pigmented. The deflection of the head to the embryo's right, as a re-

sult of the greater development of the left neural fold, is clearly seen. The internal condition of an egg of this sort is shown in Figure 9 (egg 70). The left and larger neural fold has risen up, and, failing to meet its fellow, has bent to the floor of the neural plate, with which it has also fused. The right neural fold, rising up later, has fused with the outside of the left fold. Thus an egg with a double neurocoele has developed. Further back in the series of cross-sections the double structure was clearly seen, but forward the abnormal central partition and roof had begun to separate into loose cells.

It is apparent that the neural folds in these asymmetric eggs differed in their degree of development, in their size, and in their ac-

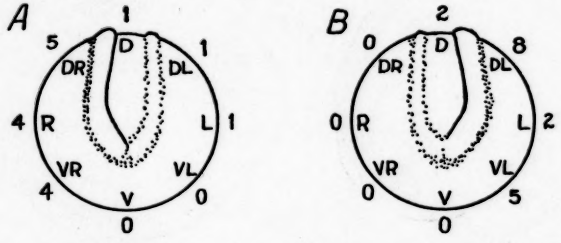


FIG. 10.—Diagram to indicate the positions of the warmed meridians in eggs with larger right neural folds (*A*), and in eggs with larger left neural folds (*B*).

tivity in neurulation. They differed in size and activity even if allowance be made for their difference in degree of development. The experimental conditions hence not only advanced the development of one neural fold, but also increased it.

In the cases just described the larger neural fold developed on the previously warmed side of the egg. This was true, with two exceptions, of the thirty asymmetric eggs of which the orientation is known (see Fig. 10). The orientation of seven eggs of this description is unknown. Three apparently were warmed exactly on the dorsal surface. The two exceptions to the principle stated (that the neural fold of the previously warmed side is the largest) were both of them somewhat ambiguous; in one case (egg 72) the warmed neural fold was largest at the anterior end, although the opposite was true farther back; while in the other case (egg 46) the smaller neural fold was associated with an accessory neural plate on the warmed side.

Occasional asymmetry was noted among the controls, but was of a different sort and capable of another explanation, as has already been noted.

In numerous instances the larger and previously warmed neural fold was darker than the other in its pigmentation; but notes as to this feature have not often been kept. In many cases the neural axis was more or less concave on the side away from the larger neural fold. This was especially noticeable in six eggs, and led in later development to a spiral curvature such that both the under side of the head and the blastopore were displaced toward the previ-

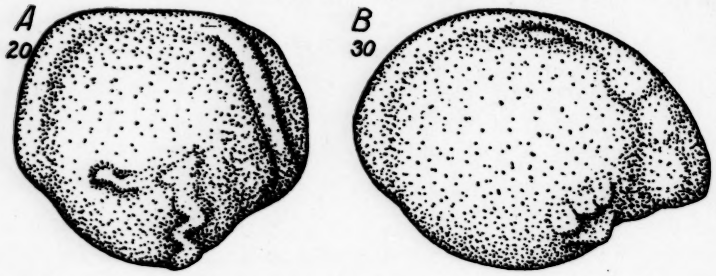


FIG. 11.—Accessory neural structures. Two examples (eggs 20 and 30) of "secondary puckering." Note that the puckering is located well toward the ventral side and away from the region of the blastopore. ×20.

ously cooled side. In a few eggs the blastopore appeared to be displaced toward the side of the smaller neural fold, apparently as a result of the greater activity of the posterior end of the larger neural fold.

E. *Accessory neural structures.*—Although the number of examples of accessory neural structures which occurred among the experimental eggs was not large, yet the abnormalities themselves were clear and are of considerable theoretic interest. In the first place, some five eggs showed puckered areas on the side in addition to more or less normal neural structures on the dorsal surface (see Fig. 11.) Since these puckerings resembled the "primary puckerings" already described, I have called them "secondary puckerings." There is reason to believe that they, as well as the primary puckerings, are related to neural folds. It will be noted that in the eggs figured (eggs

20 and 30) the puckered area is well toward the ventral side and away from the blastoporal region. This position was characteristic of all the eggs which showed secondary puckering, and has some theoretic significance in accounting for the phenomenon, as will be brought out in the discussion.

Sections through the puckered areas (see Fig. 12) show the puckerings to be solid thickenings of ectoderm, and entirely independent of the other two germ layers. In fact, in egg 20 (Fig. 12) the mesoderm had not yet reached the area; while in egg 30, which was allowed to develop to the early limb bud stage before it was sectioned, although mesodermal cells had reached the region, they had taken no part in the outgrowths. In this egg (30) the puckerings appeared as solid epidermal warts of considerable size.

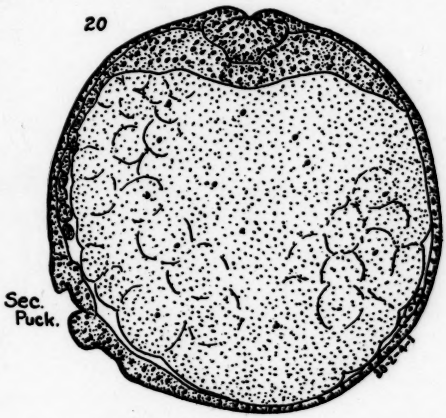


FIG. 12.—Accessory neural structures. A cross-section through the region of secondary puckering of egg 20. The puckering is purely ectodermal, the mesoderm not having as yet entered the puckered area. ×40.

In a number of eggs the secondary puckerings were clearly related to the neural folds of the same size (see Fig. 13*A*, egg 37). The fold was in each case more or less disorganized, and instead of extending backward, it bent outward so as to join and include some of the puckerings. I have tabulated the condition as "aberrant neural fold." This condition grades into that of eggs, such as 39 and 26 (Figs. 13*B* and 13*C*), in which the deflected neural fold is organized into accessory brain areas. The feature is of interest because of its bearing on the question of brain determination—a subject not discussed in this paper.

A cross-section of egg 26 is shown in Figure 14. The thickly nucleated tissue on the right is apparently ectodermal and represents

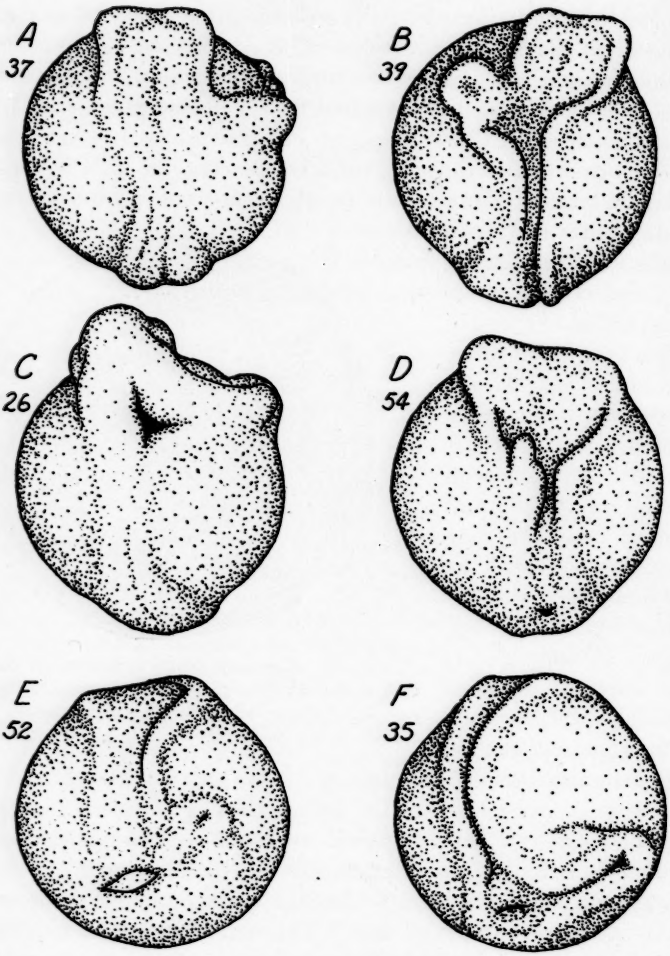


FIG. 13.—Accessory neural structures. *A* (egg 37) shows the right neural fold deflected outward toward an area of secondary puckering ("aberrant neural fold"). *B* (egg 39) and *C* (egg 26) show accessory brain areas. *D* (egg 54) shows an abnormal left neural fold with a swelling midway of its length. *E* (egg 52) and *F* (egg 35) show small accessory neural plates which have developed outward from the blastopore. ×20.

the accessory brain region. The egg at the time it was killed had ceased to progress in its development, and would soon have died. The sections indicate a considerable amount of activity in cell division, but suggest that the neural region has begun to lose in its organization. The position of what is apparently the anterior tip of the notochord can be recognized just beneath the primary neural tube, but the distribution of mesoderm is in doubt. It apparently does not extend far to the right beneath the accessory brain material. The dense mass of cells near the center of the section was united to nervous tissue a few sections farther forward.

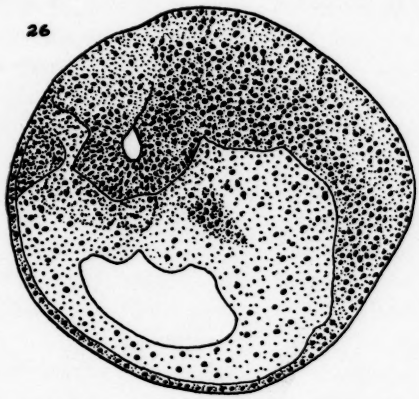


FIG. 14.—Accessory neural structures. Cross-section through egg 26 showing primary neural tube (with neurocoele) and longitudinal section through the accessory brain area (right). The egg had ceased to progress in its development at the time it was preserved; and the neural plate had begun to disorganize. ×40.

A number of examples of secondary puckering and of disorganized neural folds appeared in eggs which were disturbed before their removal from the apparatus, and of which, in consequence, the orientation with respect to the thermal gradient is unknown. The three eggs, however, of which the orientation is known, showed puckerings or disorganized neural folds on the previously warmed side. This fact, together with the fact that in the other eggs of the description the puckerings always occurred on the more darkly pigmented side, indicates that the accessory formations are related to the previously warmed area.

Of importance also are certain eggs which showed accessory neural plates extending outward from the blastopore. The condition is illustrated by eggs 52 and 35 (Figs. 13*E* and 13*F*). In the latter example there is clearly visible an accessory neural plate with anterior neural fold and two lateral neural folds. Figure 15 is a section

of this egg (35) transverse to both neural areas. It would be difficult to say from the section which of the plates, primary or accessory, is the more typically developed. Although the neural plate material and the mesoderm are difficult to distinguish, the section being close to the blastopore, yet it is probable that the mesoderm and chorda (?) have typical relations in the accessory structure.

The accessory neural plate developed on the warmed side of the egg in the two cases for which the orientation of the egg is known.

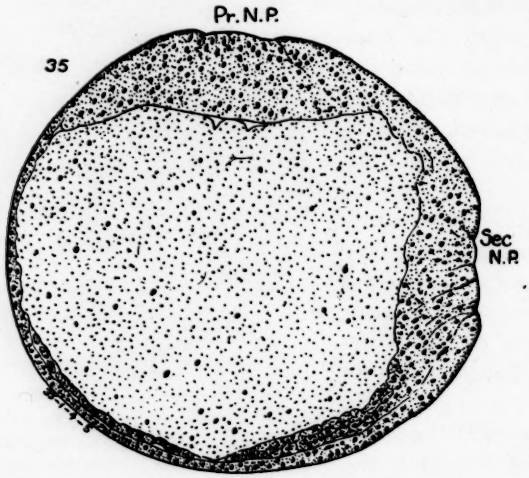


FIG. 15.—Accessory neural structures. Cross-section through the primary and accessory neural plates of egg 35. The distinction between neural tissue and chorda-mesoderm was not clear at the level drawn, because it was close to the blastopore. ×40.

Three eggs showed strange swellings midway along the neural fold (Fig. 13*D*), which for want of a better designation I have called "abnormal neural folds." The condition may be the result of a disorganized lateral area of the neural plate having closed in toward the midline. In one case the abnormality was on the previously warmed side; in two cases it was on the previously cooled side. The latter fact presents difficulty.

## DISCUSSION

The results which have just been described reveal a number of significant correlations. The interpretation of these correlations,

however, in terms of the mechanics of development, is not an easy matter. The suggestions here offered are to be taken in a tentative way. They are the conclusions which seem to the writer most satisfactory in the light of his present knowledge.

Fortunately for our purposes, the analysis of the mechanism of urodele development has made rapid progress during the past several years, due primarily to the work of Spemann, Vogt, and their associates in Germany. For the first time our knowledge of the topography of the blastula with respect to the prospective significance of its different areas has attained a fair degree of completeness (Vogt, 1925, 1926). The past few years, moreover, have shown notable advance in the disentanglement of the causal sequences of early determination. Especially important are the papers of Spemann (1918, 1921), O. Mangold (1923), Spemann and H. Mangold (1924), Geinitz (1925), Marx (1925), Bautzmann (1926), Spemann and Geinitz (1927), and Mangold and Spemann (1927). The work of Goerttler (1925, 1926) must also be considered. Advantage will be taken of this literature in interpreting the present experimental results.

A conception which has proved fruitful is the theory of physiological gradients as developed by Child. I have, however, made use of the theory only in part. I do not see that an egg, following fertilization, is axiate in its structure; rather it possesses a physiological pattern in which different regions differ in their rates of "metabolic" action. Just what the basis of this physiological pattern is I shall not attempt to say. In some fashion, I do not know just how, this physiological pattern is related to the pattern of chemical differentiation which goes on in the egg. Differences which at first are quantitative become differences which are qualitative. In this process there appears to be an interaction between the regions of high metabolic activity and adjacent regions of low activity; at any rate, the pattern of chemical differentiation is seemingly related to the direction and steepness (?) of the metabolic gradients. Later, the invisible chemical pattern which has come into being issues in differential dynamic activities and structural modifications. A suggestive paper in interpreting the physiological pattern of the amphibian egg is that of Bellamy (1919). Hyman's papers on the fish egg (1921, 1926) and hen's egg (1927) are also to be mentioned.

It is assumed, in interpreting the experimental results, that a thermal gradient actually modifies the physiological pattern of the egg and influences the invisible processes of chemical differentiation ("determination"). Warming a region of high metabolic activity presumably increases the steepness of the metabolic gradients about it, while cooling such a region levels down the gradients. In some instances localized warming may be supposed to create new centers of high metabolic activity and, hence, new gradients. Whether or not the physiological pattern as modified by the temperature gradient persists after the difference of temperature is removed has not been determined; but whether it does so or not, the modified chemical differentiation probably remains.

The deformities produced by the thermal gradient were not the results of injuries or retardations resulting from overcooling. The temperature of the cold water bath was never lower than 13° C., and the temperature at the surface of the egg was always at least 3° above this. At such a temperature the egg develops normally. Moreover, the period of the application of the thermal gradient was relatively short. In the case of the eggs now reported it fell entirely within the blastula stage. The abnormalities which were observed appeared at least two days later, and cannot, therefore, be thought of as the direct and immediate result of the temperature difference. We must think of the thermal gradient (*a*) as having in the first instance directly altered the physiological pattern of the egg; (*b*) as having in consequence modified the processes of chemical differentiation; and (*c*) as having finally and in due time affected the differentiation of visible structure. These facts seem to be necessary inferences from the conditions of the experiment.

A second fact of significance developed by the experiments is that the thermal gradient, although at first undoubtedly affecting only the rate of the processes of chemical differentiation, in the end affected also, at least in some instances, their nature and their extent. The observations indicate that the egg is not simply a mosaic of independent, self-differentiating parts which may be made to differ in their embryonic age or degree of development; but that it is also, in some degree, a correlative whole. In the case of asymmetric neural folds, the folds of the previously cooled side were not merely

younger; they were also smaller and less active, and never attained to the activity of the larger structure. In the case of accessory structures it is obvious that correlative chemical differentiation has been influenced. It would appear that, as a result of the temperature gradient, the normal integrating function inherent in the physiological pattern of the egg was interfered with.

The present observations are in accord with the view that the fundamental bilaterality of the egg is already determined soon after fertilization. There has been nothing to indicate that the thermal gradient either controlled or modified the position of the dorsal axis of the embryo. Spemann (1918) has shown that the dorsal-lip area is the first region of the egg to be irreversibly determined. Now, certain of the experiments here reported presumably modified the physiological pattern as early as the eight-cell stage. It is concluded, therefore, that the chemical differentiation by which the dorsal-lip area is determined was already well under way, if not complete, by the eight-cell stage. This conclusion agrees with the findings of Ruud (1925).

The temperature gradient may have affected the processes of gastrulation; but this is not proved, and the matter is in need of further study with better controls and with apparatus designed to permit viewing the egg from beneath.

The thermal gradient affected the processes of neural-plate formation. There is a small amount of evidence to support the belief that when a neural plate forms on the previously warmed side of the egg, it is likely to be smaller and shorter than normal. If true, these relations are the inverse of what one would expect in case the thermal gradient influenced the rate of development only. The evidence is not sufficient to justify further discussion of this matter at the present time. When the neural plate develops on a side of the egg intermediate between the thermodes (sagittal plane of egg transverse to direction of thermal gradient), an asymmetry in the development of the lateral neural folds commonly results. The fold of the previously warmed side is commonly larger and more active in the movements of neurulation. In this case we seem to be observing the effect of a thermal gradient which neither favored nor retarded the metabolic "dominance" of either the animal pole or dorsal-lip area,

but which favored the development of one side of the neural area over the other. The results may be interpreted as a consequence of an asymmetric chemical differentiation of prospective neural-plate material, which later finds visible expression in unequal neural folds. The results may be compared to those of Huxley (1926) in which he applied a vertical thermal gradient to frog's eggs. When the gradient was descending (animal pole warmed), he supposedly increased the "dominance" of the animal pole and augmented the chemo-differentiation of the anterior end. The result was an unusually large brain area. An ascending gradient produced the opposite results.

We conclude that in general a thermal gradient alters the normal metabolic pattern of the egg, and thus modifies the processes of chemical differentiation. Later, the modified chemical pattern reveals itself in the cell movements of gastrulation and neurulation, and in structural deformity.

Spemann and his associates have shown experimentally that a neural plate is formed in animal pole ectoderm when it comes in contact (or close proximity) with the chorda-mesoderm of the archenteric roof. They conclude that in normal development the determination of the neural plate is by induction through the activity of mesodermal and chordal cells derived from the dorsal-lip area. Goerttler (1925, 1926) and Lehmann (1926) on the other hand, have obtained evidence that the prospective neural-plate material is already chemically differentiated before gastrulation begins, and before any contact with chorda-mesoderm is possible. In a recent paper Spemann (Spemann and Geinitz, 1927) discusses the meaning of this apparently contradictory evidence. Determination of the neural plate, he notes, is probably not a single event, but a doubly or multiply linked chain of events. There occurs, first of all and before gastrulation begins, an early chemo-differentiation of a region of prospective neural-plate material. This early differentiation is sufficient to determine the behavior of the neural area during the cell movements of gastrulation—Vogt speaks of it as "dynamic determination"—but is partial and reversible so far as the histological fate of the region is concerned. Spemann suggests that even this early determination may be a result of the relation of the neural area to the prospective mesoderm of the future dorsal lip. We may

think of such a relation in terms of the high physiological activity of the dorsal-lip area.

Later, after gastrulation has taken place, there occurs, as Spemann and his associates have abundantly shown, a further and irreversible determination. This time it is by induction through proximity of the prospective neural-plate area to the mesoderm and chorda which now form the archenteric roof. Again, this induction may well be a consequence of the high developmental activity of the chorda-mesoderm, an interpretation borne out by the fact that other highly active materials, namely, the optic region of a neural plate, are also able to induce the formation of a secondary neural plate (Mangold and Spemann, 1927).

These concepts raise a problem with regard to the present experimentation: Does the thermal gradient produce abnormalities of the neural plate directly, by affecting the processes of chemical differentiation of prospective neural-plate material at the early determination; or does it act indirectly and upon the second determination, by influencing the chemical differentiation or the migration forward of the mesoderm which comes to underlie the plate? Does the gradient affect the first determination of the neural plate, or the second; or does it affect both? At first I was inclined to the second alternative. Observations of Brachet (1923, 1927) on the frog's egg and of Lehmann (1926) on the egg of *Triton* showed that lateral injury or deficiency of the mesoderm of the dorsal-lip area leads to deficiency of the neural fold of the injured side. This suggests that the effect of the thermal gradient may be to cause an asymmetric development of mesoderm, and affect the neural plate at the second determination.

An analysis of the abnormalities which I have called "secondary puckering" and accessory neural structures, however, has led me to favor the first alternative and to suppose that the thermal gradient affects the early determination of prospective neural-plate material. I have interpreted "primary puckering" as the result of an unorganized attempt of prospective neural-plate material to form neural folds. Whether or not chorda-mesoderm underlies the region of such puckering has not been determined. It probably does not, or is at any rate deficient under the region, since puckering of the sort is

always associated with difficulties of gastrulation. It seems probable that primary puckering occurs when the chemical differentiation of the first determination has taken place, but the mesodermal induction of the second determination has been defective. The organization of the region of prospective neural-plate material into an equatorial neural fold, such as was shown by egg 86 (Fig. 5*C*) and such as is illustrated in some of Goerttler's (1926) figures, probably occurs when the cell displacements of gastrulation are incomplete, but the region of prospective neural-plate material is not altogether free from the influence of mesoderm.

I have interpreted "secondary puckering" as evidence for the existence in the lateral ectoderm of a secondary area with the potentialities of prospective neural-plate substance. We may think of the origin of such an area in terms of physiological gradients. Just as primary puckering is evidence of the presence of prospective neural-plate material which has formed from ectoderm in relation to the metabolic activity of the animal pole (?) and dorsal-lip region; so secondary puckering may be taken as evidence of a secondary region of the same sort which has undergone determination in relation to the high rate of metabolism of the heated side of the egg. In the one case, the determination is a chemical differentiation related to the normal metabolic pattern of the egg; in the other case, it is a chemical differentiation related to the pattern induced by the thermal gradient. This interpretation rests on a small amount of evidence. If, however, it be sound, it indicates that a thermal gradient applied during cleavage affects directly the early determination of prospective neural-plate substance.

The organization of a region of secondary neural-plate material into a neural plate is probably conditioned on its coming into contact or close proximity with chorda-mesoderm. The relation may well be a result of the high rate of developmental activity of the latter tissue. The view is suggested by the study of certain eggs of the present series (cf., for example, Figs. 12 and 15). The accessory neural plate of egg 35 (Fig. 15) is underlaid by mesoderm; on the other hand, the secondary puckering of egg 20 is entirely independent of mesoderm (Fig. 12). What portion of the accessory brain area of egg 26 is underlaid by mesoderm is uncertain (see Fig. 14);

apparently but little. Spemann (Spemann and Geinitz, 1927) recognizes that the forebrain area of the neural plate is not in immediate contact with mesoderm, although its organization is clearly conditioned on continuity with the rest of the neural plate, and hence on proximity to mesoderm.

If these principles are valid, the fate of a secondary neural area varies according to its location with respect to the other regions, presumably the most active regions developmentally, of the egg. (1) If the region of secondary neural material is near the blastopore, it will be entered by mesoderm from the blastopore and organized under its influences into an accessory neural plate. (2) If such a region is removed from the blastopore but near the dorsal axis, it will come under the influence of the mesoderm of the archenteric roof and of the primary neural plate, and organize as an aberrant neural fold or accessory brain area. (3) If, however, it is removed from both these regions, i.e., if it is forward and ventral in position, it will not come under the influence of the more active areas and will form a disorganized puckered area.

These relations are illustrated in the schematic Figure 16. The first vertical column shows four eggs at the beginning of gastrulation. In each case the animal pole is shown as upward, and the first sign of invagination represented by a horizontal line. The areas striped diagonally indicate prospective neural-plate substance—primary neural substance in case the striping is downward to the left, secondary neural substance if it is downward to the right. The region striped horizontally is prospective notochord. The region dotted most densely is prospective mesoderm of the future somites; that dotted less densely prospective mesoderm of the splanchnopleure and tail bud. The first egg of the column (1*a*) shows the normal condition according to the work of Goerttler (1925) and Vogt (1926). The second egg (2*a*) is similar to the first, but shows in addition a region of secondary neural material adjacent to the mesoderm of the blastoporal lip. The third egg (3*a*) has a secondary region of the sort adjacent to the primary neural area, while the fourth egg (4*a*) has a secondary region isolated from both the mesodermal and primary neural regions. The second column of the figure shows the same four eggs at a later stage in gastrulation. The arrows in-

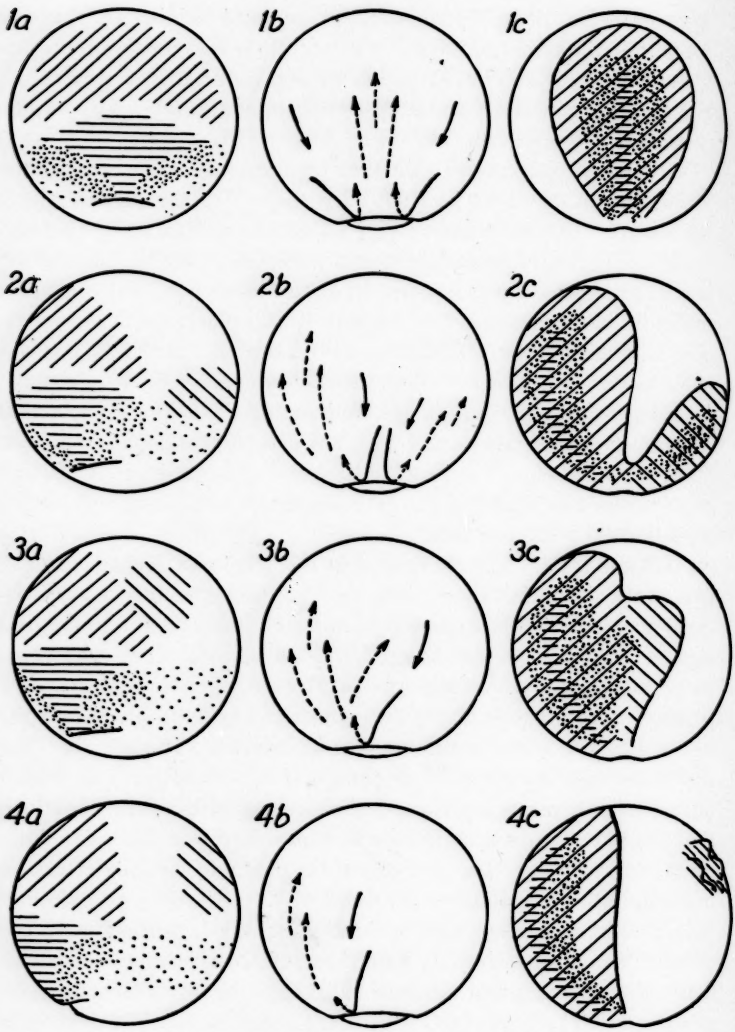


FIG. 16.—Schematic diagram to indicate the fates of blastular areas in normal and in certain experimental eggs. For explanation see text.

dicate the direction of the cell movements which presumably are in progress. The movements shown for the first egg (1*b*) are based on the work of Goerttler and Vogt. The third column represents the same four eggs at the close of the period of gastrulation. The mesoderm and chorda have passed beneath the dorsal blastoporal lip and form the archenteric roof beneath the neural plate. The prospective neural-plate material has stretched toward the blastopore and formed the definitive neural structure. The first egg figured (1*c*) shows the normal relations of the regions. The second egg (2*c*) shows primary and accessory neural plates, both of them underlaid by mesoderm. The condition is that found, for example, in egg 35 (Fig. 13*F*). The third egg (3*c*) shows the condition which I have called "aberrant neural fold," or accessory brain area (see Figs. 13*A*, 13*B*, and 13*C*). The extent of mesoderm in this case is uncertain, but that the area is under the influence of dorsal mesoderm and the primary neural plate is evidenced by the continuity of neural structures. The last egg (4*c*) shows secondary puckering (Figs. 11 and 12). There is in this case no mesoderm nor other region of high developmental activity in the neighborhood of the secondary neural area.

The abnormality called "accessory neural plate," which occurred three times, raises the question as to whether prospective neural-plate material in some way influences the forward migration of mesoderm. If the presence of chorda-mesoderm beneath the neural plate is solely a coincidence, dependent upon the regularity of the cell movements of gastrulation, a result of the general orderly nature of developmental processes, then the presence of mesoderm beneath the accessory neural structure is unaccounted for.

## SUMMARY

1. The experiments here reported involved the application of a horizontal temperature gradient to the eggs of the Pacific Coast newt, *Triturus torosus* (Rathke).

2. An apparatus was devised consisting of a warm and a cold water bath, and of pairs of aluminum plates or "thermodes." The latter were used to conduct heat to and from opposite surfaces of rows of eggs. Thin agar jelly (one-fourth of 1 per cent) was used as

a medium about the eggs, to prevent convection and to support the eggs in place.

3. A horizontal temperature gradient of about 5° C. was maintained for forty hours between opposite surfaces of eggs at the blastula stage.

4. The most outstanding results noted were the following:

*a*) A decided tendency for the neural fold to be larger, darker, and more active on a previously warmed lateral side, when the gradient was transverse.

*b*) The occasional development of secondary neural structures on a previously warmed lateral side, ranging all the way from irregular "puckerings" to aberrant neural folds, accessory brain areas, and accessory neural plates.

5. Among the conclusions suggested are the following:

*a*) The thermal gradient probably acts by affecting directly the physiological pattern (metabolic gradients) of the egg, and indirectly the processes of invisible chemical differentiation. Later visible structural changes appear.

*b*) The thermal gradient affects not only the rate but also the position and extent of chemical differentiation.

*c*) The thermal gradient has not altered the fundamental bilateral symmetry of the egg. This was probably irreversibly determined before the experiments began.

*d*) The thermal gradient has, however, notably affected the development of the neural plate, probably by influencing the physiological pattern of the egg at the time when prospective neural-plate material is first determined. The instances in which the warmed side was dorsal or ventral were too few to justify conclusions. When, however, the warmed side was lateral, asymmetry in the differentiation of the neural-plate material occurred, and unequal neural folds resulted.

*e*) Warming one side of the egg occasionally produced secondary areas of prospective neural-plate material, related probably to the high metabolism of the warmed area in the same manner as the primary neural-plate material is related to the high metabolism of the future dorsal-lip region. This secondary neural area later revealed itself in accessory neural plates, accessory brain areas, or in irregular "puckerings." The organization of such an area into rec-

ognizable neural structures was apparently conditioned on proximity to regions of high developmental activity; namely, to the chorda-mesoderm of the archenteric roof or to the primary neural plate.

*f*) The question as to whether prospective neural-plate material influences the migration forward of chorda-mesoderm during the process of gastrulation is raised.

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# THE EFFECTS OF CERTAIN ANESTHETICS ON THE CLEAVAGE REACTION IN SEA-URCHIN EGGS

(Seven figures)

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IN A recent paper (Blumenthal, 1927) it was shown that cyanides, although being themselves anesthetics or producing anesthesia, may behave differently from many other anesthetics. Lillie (1918) contends that anesthetics in general produce a decrease in permeability,[1] whereas the author has shown that KCN did produce a decrease in the permeability of *Arbacia* eggs to water, but HCN produced, instead, an increase in permeability. No attempt at an explanation was made at that time.

The amount of work done with anesthetics and on anesthesia in general is great, and numbers of theories tending toward an explanation of this important physiological process have been advanced. Winterstein gives an excellent review of the subject in his recent book (1927). But exceptions, and important ones, to almost all of these theories have been found, and one is inclined to believe that the problem is far from being solved.

It is generally conceived that anesthesia is a condition in which the vital processes of the cell have been inhibited, and that this condition must be reversible. If anesthesia, as here defined, is prolonged and the condition is no longer reversible, then the cell is dead. According to current definitions, both metabolism and growth or cell division must be reversibly inhibited during anesthesia. But basic metabolism in many cases of anesthesia is not inhibited. In fact, the objections to Verworn's asphyxiation theory of anesthesia (1911) have been based on experiments which showed that many anesthetics in dilute concentrations increase instead of decrease oxidation (Lillie, 1916; Hyman, 1919; Buchanan, 1923; Tashiro and Adams,

[1] Cf. R. S. Lillie, *J. Biol. Chem.*, XVII (1914), 120, for sea-urchin eggs.

1914) or do not affect oxidations at all (Loeb and Wasteneys, 1913). In many cases the criterion for anesthesia has been the reversible inhibition of cell division. If cell division is inhibited, but oxidation processes not, does that condition constitute anesthesia under the old definition? Those who have attempted to explain anesthesia have assumed that anesthesia in all cases is similar and that all anesthestics act alike, producing the same condition in the same manner. The fact that exceptions have been found to all attempted explanations seems to indicate that there may be several types of anesthesia and that all anesthetics may not act in a similar manner.

Heilbrunn (1925), Höfler and Weber (1926), Krehan (1914), and Blumenthal (1927) have shown that certain anesthetics produce an increase in permeability, all in contradiction to Lillie's permeability theory of anesthesia (1916). It would seem likely that either several different types of anesthesia are being produced or that the same condition has been brought about in entirely different ways. Which of the two statements is nearer correct is doubtful at present. If the types of anesthesia are unlike, then methods other than those depending on cell division as the criterion of anesthesia must be employed. Cell division may be inhibited in all cases, but the effect upon other internal processes may vary. If anesthesia is fundamentally alike in all cases, but brought about differently by different anesthetics, the attempts to propound one general explanation for this phenomenon seems unjustified.

In the experiments previously mentioned (1927), it was found that eggs anesthetized with HCN and KCN could be fertilized while they were in that condition. This rather surprising fact was noted but no further work done on it until this last summer. When this work was continued using as anesthetics KCN and several other chemicals which have been used by Lillie (1913), it became evident immediately that the action of KCN varied greatly from the action of the other anesthetics used.

The anesthetics employed were as follows: 5 per cent methyl cyanide; 1.5 per cent ethyl acetate; 3 per cent iso-propyl alcohol; 10 per cent methyl alcohol; 3 per cent n-propyl alcohol; and KCN varying in concentrations from $N/100$ to $N/1{,}000$. These anesthetics

were chosen because of their variety and because they were the only ones, out of a number tested, which would inhibit cell division of fertilized *Arbacia* eggs for ten hours or more. Their concentrations, except for that of the KCN, were those found by Lillie to give the optimum anesthetic condition (1913). Only the concentrations of the KCN were varied.

METHOD

All the solutions were made up in normal sea water and kept in glass-stoppered bottles. The pH of the solutions was measured colorimetrically and found to range from pH 6.8 to pH 9.2. No attempt was made to bring the solutions back to the pH of normal sea water, but controls were run at the same hydrogen-ion concentration as the experimental solutions. It was found that exposure for one hour of fertilized and unfertilized *Arbacia* eggs to sea water having these hydrogen-ion concentrations had no effect on the subsequent time for first cell cleavage. Thereafter only one control in normal sea water was run.

Unfertilized *Arbacia* eggs, usually from a single individual, were exposed to the various anesthetics, and at ten-minute intervals some eggs were pipetted into normal sea water and inseminated. It was not found necessary to wash the eggs before insemination, because the amount of liquid taken up with a drop of eggs was less than 0.05 cc. and this was transferred to 15 cc. of sea water. The time for 50 per cent of the eggs to reach the first cleavage stage was recorded and the eggs then discarded. A stop watch was used to record time intervals and the eggs were observed under a binocular which gave a magnification of 50×. Between one hundred and two hundred eggs were under observation simultaneously. Six readings were obtained of the cleavage time for eggs which had been exposed to the anesthetics for ten, twenty, thirty, forty, fifty, and sixty minutes, respectively. The same method was repeated with fertilized eggs. In this case the eggs were first inseminated in sea water and permitted to stand for four to six minutes, by which time the fertilization membranes were fully formed. The eggs were then transferred to the anesthetic solutions and removed at ten-minute intervals. The time for first cleavage of 50 per cent of the eggs after removal from the anesthetics was recorded and the four to six minutes added

to that time. Controls in sea water of pH 6.8, 8.2, and 9.2 were run at the same time. Room temperature was recorded before and after each experiment and was found to vary about $\pm 0.5^{\circ}$ C. during the course of a single experiment.

The same procedure was followed, running both fertilized and unfertilized eggs side by side, and comparisons made. The time for 50 per cent of the experimental eggs to reach the two-cell stage was subtracted from the time for 50 per cent of the control eggs to show first cleavage, or vice versa as the case might be, and the difference plotted against the concentration of KCN or other anesthetic used.

## RESULTS

In the case of both fertilized and unfertilized *Arbacia* eggs, the length of exposure to the anesthetics had no effect in increasing the depth of anesthesia of the eggs, if the measure of depth of anesthesia was the time for first cleavage to occur. Eggs exposed to the anesthetics for sixty minutes came out of anesthesia in the same time that eggs exposed for only ten minutes did. Exposures of ten hours' duration only slightly increased the time for first cleavage, and this occurred only because the eggs had been injured. This injury became evident since only abnormal division took place when the eggs were inseminated after the long exposures.

The failure of length of exposure to affect depth of narcosis can be seen from Figure 1. Two separate experiments are recorded. Along the ordinate are plotted the differences, in minutes, between the time for first cleavage of the exposed eggs and the control eggs. Along the abscissa are the concentrations of KCN and the six ten-minute readings for each concentration, at which periods eggs were removed from the anesthetic solutions and fertilized. The short lines have been drawn through the six points representing exposures of from ten to sixty minutes to each concentration of KCN. The fact that there is only a slight irregularity, due no doubt to experimental error, and not a consistent increase or decrease in time for cleavage to occur, is taken as showing that length of exposure did not delay the time for first cleavage. The heavy continuous line has therefore been drawn through the averages of these six readings represented by the crosses. From the manner in which these graphs have been

plotted, the time for first cleavage of the control eggs to occur is represented by the zero along the ordinate.

It is also seen from this figure that unfertilized eggs anesthetized by KCN do not come out of anesthesia immediately after removal

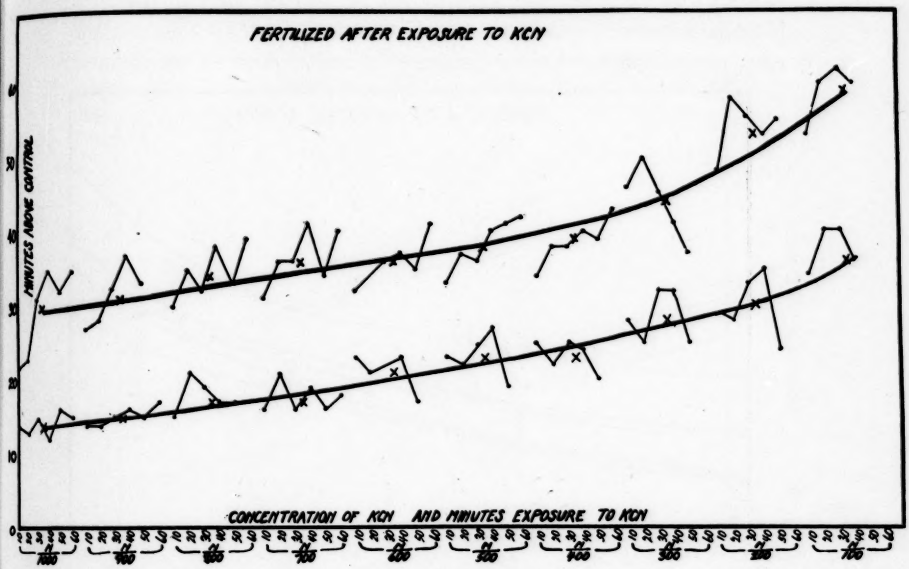


FIG. 1.—The ordinates are differences, in minutes, between the time for first cleavage of the exposed and control eggs. The abscissa shows the concentrations of KCN and the six ten-minute readings obtained for each concentration. The short lines have been drawn through the six points representing exposures of from ten to sixty minutes, while the continuous heavy lines have been drawn through the averages of each set of six readings. Two experiments are here recorded. The zero along the ordinate, therefore, is the time when cleavage in the control eggs occurred. Eggs were fertilized after exposure to the cyanide.

from the anesthetic. The eggs were inseminated immediately after removal from the anesthetic, and fertilization membranes were formed as usual. Cell division, however, is delayed, the length of delay depending upon the concentration of KCN, but seemingly independent of the length of exposure to these concentrations. The difference between the two experiments recorded in Figure 1 is due not only to

the difference in temperature at the time they were performed, but also to the difference in the maturity of the two lots of eggs.

Figure 2 shows the same thing for a greater number of experiments. In this figure, all points are the averages of six ten-minute readings for each concentration of KCN. All the eggs in these experiments were fertilized after exposure to KCN. In Figure 3 the eggs were fertilized before exposure and after four to six minutes

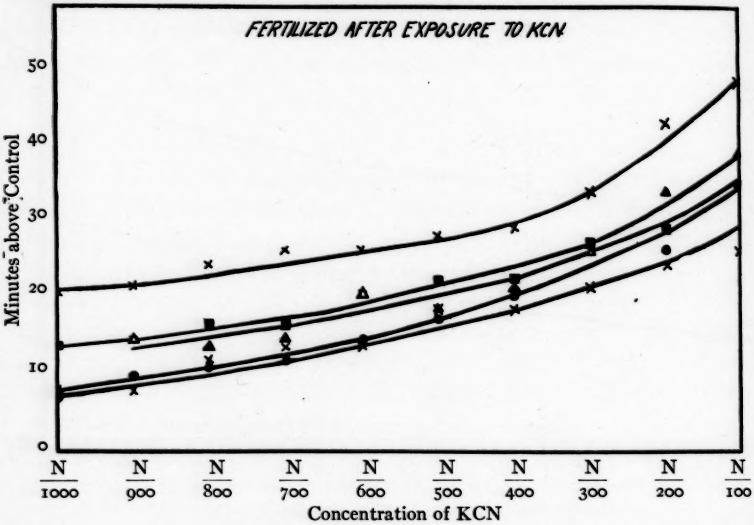


FIG. 2. Same as Fig. 1. A greater number of experiments are shown, and all points are averages of the six ten-minute readings for each concentration.

transferred to the KCN solutions. These four to six minutes were added to the length of time for first cleavage to occur after removal from the KCN.

In Figure 4 are recorded the results obtained when fertilized and unfertilized eggs were run side by side. The points are averages of six ten-minute readings, and the continuous line represents eggs fertilized before exposure while the broken lines represent eggs fertilized after exposure to the KCN. Both fertilized and unfertilized eggs show a delay in cell division after exposure to the KCN, the fertilized eggs showing a greater delay than the unfertilized. This cor-

roborates the work of Loeb (1906) who showed that fertilized eggs are more susceptible to cyanides than are unfertilized. Loeb took percentage of development as the criterion of susceptibility.

All the foregoing results were obtained when using KCN as the anesthetic. The results with the other anesthetics are entirely different. Figure 5 represents two separate experiments. The ordinates

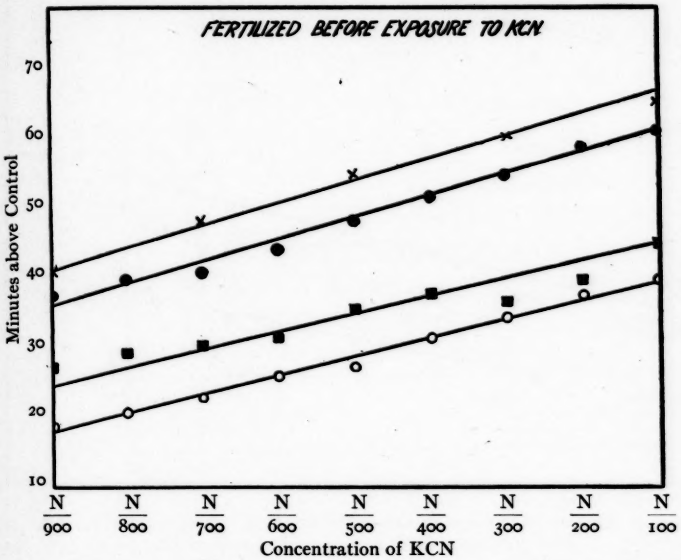


FIG. 3.—Same as Fig. 1, but eggs were fertilized before exposure to KCN

are the differences in time for first cleavage after removal from the anesthetic between experimental and control eggs, and the abscissae are lengths of exposure to the anesthetics. Eggs were fertilized after exposure. Instead of finding a delay in cell division as was found with KCN, here we find the eggs, after exposure to the anesthetics, dividing in the same time, within experimental error, as the control eggs do. The zero line in the graph represents the time when division of the control eggs occurred. Even methyl cyanide, which was included in this series of experiments, behaved as the alcohols and not as KCN. Fertilization membranes were formed immediately after in-

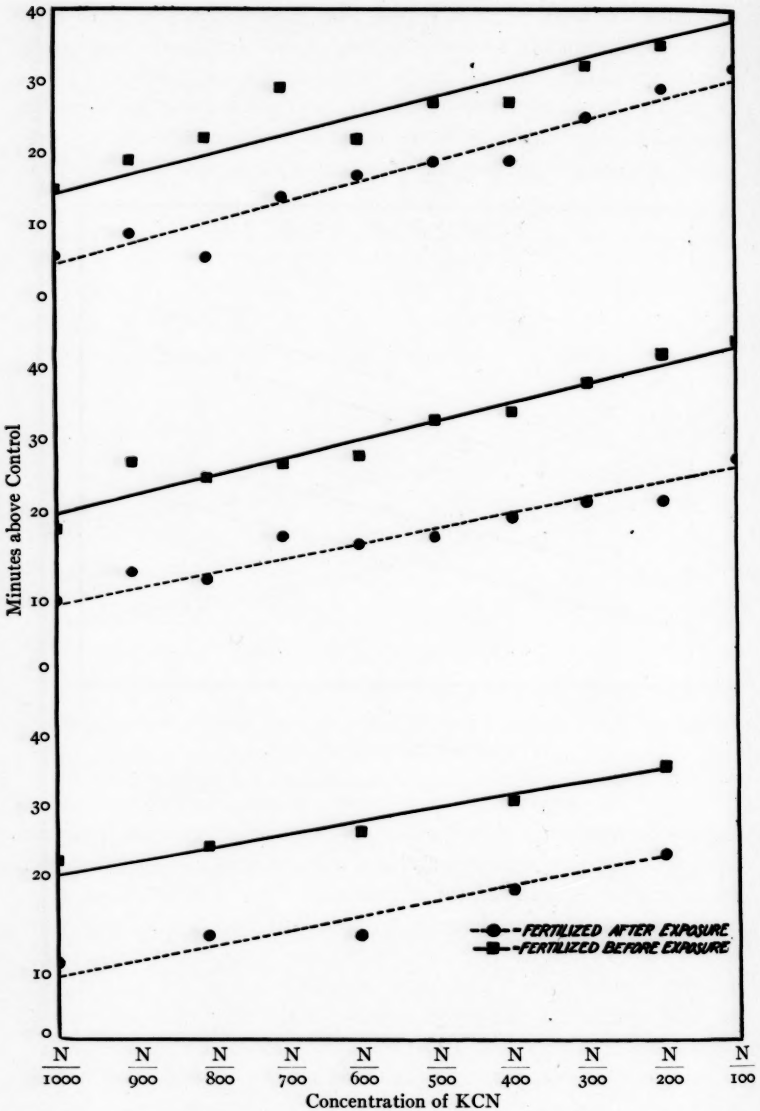


FIG. 4.—A comparison between eggs fertilized before and after exposure to KCN. Three sets of experiments are represented.

semination. It is also evident that the length of exposure did not affect the time for first cleavage.

Eggs fertilized before exposure to the alcohols show even a slight hastening of cell division beyond that of control eggs, as shown in Figure 6. Here again length of exposure fails to show any specific effects. In Figure 7 is shown a comparison of the effects of these

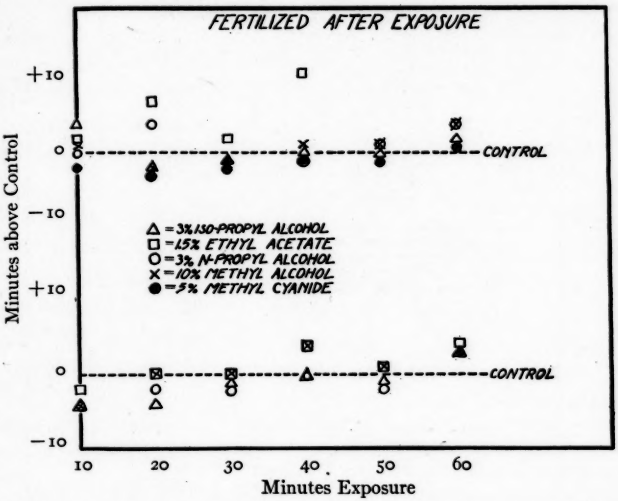


FIG. 5.—Along the ordinate are differences, in minutes, between the time for first cleavage of exposed and control eggs. The abscissa shows length of exposure. Eggs were fertilized after exposure. Two separate experiments are shown.

anesthetics upon both fertilized and unfertilized eggs. Two separate experiments are shown, although more were done but have been omitted to avoid confusion. The points are averages of six ten-minute readings. Here again the fertilized eggs are more susceptible to the anesthetics but this suceptibility is shown by a decrease in time for first cleavage rather than an increase as was the case with KCN.

In the experiments reported above, the eggs were fertilized either before or after exposure to the anesthetics. In another series, sperm was added to the eggs while they were in the anesthetic solutions. The sperm was not anesthetized as were the eggs, but continued

to move about as in the control, for at least ten minutes. Fertilization membranes were formed immediately after introduction of the sperm, except in the case of ethyl acetate, but cell division never occurred as long as the eggs remained in the anesthetic solutions.[1] This would seem to indicate that the anesthetized eggs can be fertilized while in that condition and also that the sperm were more re-

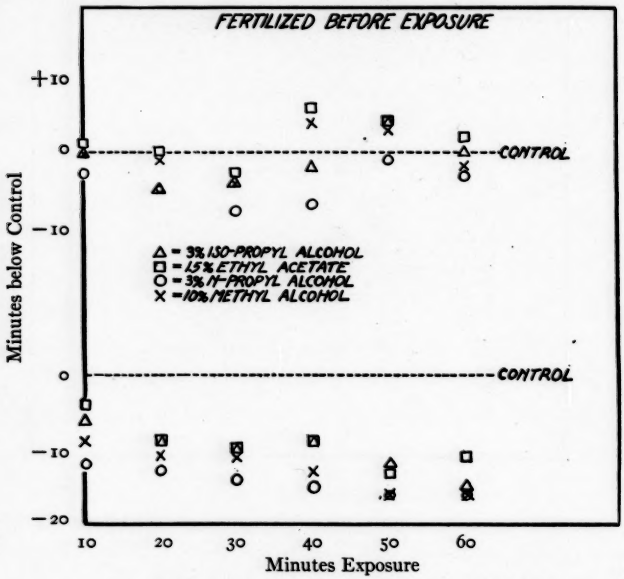


FIG. 6.—Same as Fig. 5, for eggs fertilized before exposure

sistant to the anesthetics than were the eggs. Loeb (1892), in working upon the effect of hypertonic sea water on cell division, found that the sperm could not fertilize the eggs while in the hypertonic solutions. According to him, the eggs were fertilized only after removal to sea water, at which time the sperm became motile again. A phenomenon of this kind was observed with the ethyl acetate.

If unfertilized eggs were placed in 1.5 per cent ethyl acetate and sperm added to the solution, the sperm surround the eggs, but no

[1] Anesthetics do not affect artificial membrane formation by fatty acids; cf. R. Lillie, Jour. Exp. Zoöl. XVI (1914), 591; cf. p. 608.

fertilization membranes were formed, even after nine hours. If the eggs were then transferred to normal sea water, fertilization membranes were formed immediately and many sperm became motile again. These eggs then continued to develop. Whether the eggs had been fertilized before or after removal from the anesthetic solution could not be determined since it was impossible to see whether the sperm became motile before or after the formation of the fertilization membrane. Sperm in normal sea water were dead after six hours, which seems to indicate that sperm can be anesthetized.

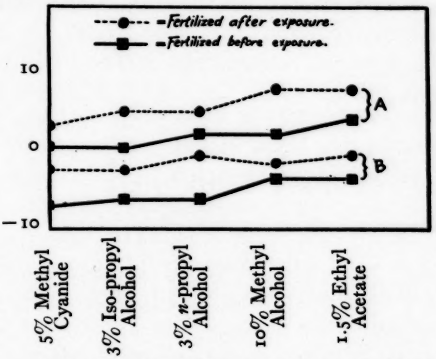


FIG. 7.—A comparison between eggs fertilized before and after exposure to the anesthetics. Two separate experiments are shown. The ordinate is difference, in minutes, between time for first cleavage of exposed and control eggs. The zero along the ordinate represents time for control eggs to cleave. The points are averages of six ten-minute readings obtained for each anesthetic.

The inhibition of fertilization membrane formation by ethyl acetate is comparable to the same sort of inhibition also demonstrated for $CuCl_2$ by Lillie (1921) and for other metallic salts by Hoadley (1923). They explain this phenomenon by the fact that the salts check the activation of the fertilizin within the egg in spite of the fact that the sperm enters the egg.

Although cell division never occurred while the eggs remained in the anesthetic solutions, even after ten hours, a certain amount of internal development was noticed in these eggs. In the control eggs, before cell division occurs, a clear zone is noticed across the center of the eggs. Subsequent cell division occurs along this cleavage zone. Eggs which were fertilized and then transferred to the anesthetic solutions showed, after a time, a similar cleavage zone. But as has been said, cell division never occurred while the eggs were in these solutions. It would seem likely, then, that in spite of being anesthetized, the eggs were undergoing some sort of internal de-

velopment. That this development is concerned with the mitotic processes has already been suggested by Loeb (1892). He found that in hypertonic sea water fertilized *Arbacia* eggs do not divide, but that the nucleus does divide. Heilbrunn (1920) also finds this with KCN solutions.

That the action of KCN is immediate is shown by the fact that eggs exposed to the various concentrations for only one minute showed a subsequent delay in cell division.

## DISCUSSION

The fact that varying the length of exposure to the anesthetics showed no specific effect upon the time for cell cleavage seems to indicate that the action is a surface action only. Once the equilibrium condition is reached, length of exposure no longer has any effect although differences in concentration do. It is possible that this is the case with the alcohols which are known to be so very surface active. But KCN must penetrate into and affect the interior of the cell since cell division is delayed until the narcotic condition has been reversed or until the narcotic has diffused out of the egg. The penetration of HCN into cells in the form of undissociated molecules has already been demonstrated by Bodine (1924) and Brinley (1927). These authors also show that solutions of KCN always contain some HCN molecules, and it is likely, if KCN does not penetrate, the HCN in the solution would. The author (1927) has already suggested that it may be the HCN molecules present in a KCN solution which gives to KCN its anesthetic properties. The failure of length of exposure to alcohols and to KCN to bring about specific effects have also been mentioned by Baldwin (1920) and Buchanan (1925), respectively.[1]

It is well known that KCN in the concentrations used inhibits cell oxidations, and this is borne out by the delay in cell division which it caused, as has been shown above. A number of workers (Warburg, 1908; Shearer, 1922; Loeb and Wasteneys, 1915) have demonstrated that there is a marked increase in oxygen consumption immediately after insemination. It is natural to assume that

[1] In a paper which has just appeared, Haywood (1927) describes similar results obtained when using $CO_2$-saturated sea water as an anesthetic. Exposures up to eighty minutes were found to cause little, if any, delay in time for first cleavage of *Arbacia* eggs after removal from the anesthetic solutions.

KCN inhibits this increase and therefore fertilized eggs should be more affected by KCN than unfertilized eggs. This has already been shown to be the case, since fertilized eggs showed a greater delay than unfertilized eggs in cell division after exposure to the KCN. Since the other anesthetics used did not cause a delay in cell division after removal from the solutions and since the eggs fertilized before exposure divided even sooner than the control eggs, it would seem to indicate that not only are the alcohols not inhibiting cell oxidations but instead stimulating it. It has already been pointed out that many anesthetics in certain concentrations increase oxidation. If the alcohols are inhibiting oxidations, it is reasonably certain that at least they are not building up any metabolic debts as did the KCN.

It is conceivable that KCN causes two distinct reactions simultaneously: the first that of inhibiting cell division (anesthesia), and the second, inhibition of oxidation and the building up of a metabolic debt which is demonstrated by the subsequent delay in cell division. That the anesthesia produced by KCN is not dependent upon the inhibition of oxidation processes by this salt and that this inhibition is a secondary result or entirely independent of anesthesia is thought to be the case by Lillie (1916). He cites instances in which it has been shown that during anesthesia the normal resting oxidation of tissues continues uninterruptedly.

> The insensitivity of many cells and tissues to simple abstractions of oxygen or the presence of cyanide solutions is in striking contrast to their sensitivity to other anaesthetics. Nerve trunks only gradually lose irritability in cyanide solutions of considerable concentrations while the desensitizing effects of anaesthetics are rapid and complete. That the two effects are essentially different is further shown by the difference in the rate of recovery which is more prompt in the case of anaesthesia.

This latter statement is borne out by the results already recorded in this paper.

Just (1924) reports some work done on *Nereis* eggs which were inseminated in approximately $N/50$ solutions of NaCl, KCl, $MgCl_2$, and $CaCl_2$. The eggs were transferred to sea water at intervals up to eighty minutes and were found to develop to the swimming stage, the percentage developing depending upon the length of exposure. No cleavage occurred while the eggs were in the solutions,

but upon removal the time for first cleavage was the same as that of eggs inseminated in sea water at the time the eggs were removed from the solutions. The same kind of reaction has been shown to occur with the anesthetics used above, except KCN. Smith and Clowes (1924) also report that $CO_2$ at a definite tension represses cell division completely. This effect is attributed to the free $CO_2$ rather than variation in hydrogen-ion concentration.

Heilbrunn (1920) has suggested that there are two types of anesthesia. This suggestion was based upon the effects of various anesthetics on the viscosity of *Arbacia* eggs. He has shown that in most cases anesthesia is accompanied by an initial decrease in viscosity. KCN, on the other hand produced an initial increase in viscosity, which condition was reversible. The present results agree with his, in view of the fact that it has been shown that the action of KCN is just the reverse of the other anesthetics employed. It is interesting to note that Heilbrunn found methyl cyanide to act as do the alcohols and not as the KCN. This same thing has already been shown in the preceding experiments. According to him, anesthesia can be explained on the basis of viscosity changes (1920).

It has been shown that the anesthetics used do not prevent the formation of the fertilization membranes, and that even though the eggs were in a condition of anesthesia, fertilization membranes form immediately after insemination. This would seem to indicate that the process of fertilization membrane formation and cell division are processes of entirely different natures. The two processes are not independent of each other, since cell division cannot occur without fertilization and therefore membrane formation, yet we find that one is and the other is not inhibited by anesthetics and anesthesia.

A number of investigators (Hyman, 1923; Just, 1919; Heilbrunn, 1913; and others) have shown that the fertilization membrane is not a formation but rather an elevation. According to Hyman, "The vitelline membrane is identical with and is elevated as the fertilization membrane." One might assume, then, that this membrane elevation is a mechanical process as compared to various internal processes and changes, and being mechanical in nature rather than physiological, is not inhibited by anesthetics or during anesthesia. In the same sense, cell division might be thought of as a mechanical

process. According to Spaulding (1904), there is a difference in potential between the osmotic pressure and surface tension of the cell, and cell cleavage is the equilibration of this difference. It might seem, then, that anesthetics should not inhibit cell division if cell division is a mechanical process. But anesthetics do not inhibit cell division directly. Their effects are rather on the processes, whatever they may be, which culminate in cell division.

## SUMMARY AND CONCLUSIONS

1. The following were used as anesthetics: $N/100$–$N/1{,}000$ KCN; 5 per cent methyl cyanide; 1.5 per cent ethyl acetate; 3 per cent iso-propyl alcohol; 3 per cent n-propyl alcohol; 10 per cent methyl alcohol.

2. Anesthetized *Arbacia* eggs can be fertilized.

3. Fertilized and unfertilized *Arbacia* eggs exposed to the various concentrations of KCN and then transferred to normal sea water show a delay in cell division above that of control eggs, the amount of delay depending upon the concentration of KCN used but independent of the length of exposure to these concentrations.

4. Fertilized eggs are more susceptible to KCN than are unfertilized eggs. This susceptibility is demonstrated by the greater delay in cell division which occurs in the case of fertilized eggs.

5. Fertilized and unfertilized *Arbacia* eggs exposed to the alcohols, methyl cyanide, and ethyl acetate exhibit no delay in cell division upon transference to normal sea water.

6. Fertilized eggs are more susceptible to these anesthetics also, but the susceptibility is shown by a hastening in cell division beyond that of control eggs.

7. Length of exposure of fertilized and unfertilized eggs to these anesthetics had no specific effect upon time for first cleavage to occur.

8. All the anesthetics employed, except ethyl acetate, do not inhibit the formation of fertilization membranes.

9. Sperm are more resistant to the anesthetics than are eggs, but they can also be anesthetized.

10. Even though the eggs are in a condition of anesthesia, a certain amount of internal development can be seen. This development is no doubt associated with mitotic processes.

11. It is suggested that KCN causes two distinct reactions simultaneously: the first, that of inhibiting cell division (anesthesia), and the second, that of inhibiting cell oxidation. It is indicated that anesthesia is not the direct result of the inhibition of oxidation.

12. A distinction is made between the process of fertilization membrane elevation and the processes leading up to cell division. The former, which is not inhibited by anesthesia, is thought of as a mechanical process and the latter as a physiological process.

The author is indebted to Dr. J. H. Bodine for valuable suggestions and criticism.

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# THE INFLUENCE OF THE GONADS ON THE PLUMAGE OF SEBRIGHT BANTAMS

(Seven plates)

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OWING to the striking sexual dimorphism exhibited by the adults, common domestic fowl have been fruitful material for investigation of the problems arising from the peculiar influence exerted upon the entire body by the primary sex organs. The use of birds, however, has called attention to a number of interesting situations that must be analyzed before any generalizations can be made. Such a situation offers the subject for the work here to be reported.

The Sebright bantam is one of the races of domestic fowl in which the plumage of the adult male is of the henny type. In the ideal cock the tail is short and square and the tail coverts are short, blunt, and but slightly curved (Plate I, Fig. 1), as in the female (Plate II, Fig. 5). Occasionally the two median tail feathers extend beyond the others (Plate I, Fig. 2). A discrepancy not to exceed an inch and a half is allowed in the breed by the American Standard of Perfection. There are no true hackle feathers on either the neck or the saddle. All the wing coverts are of the same pattern and shape, save for size. Feathers of the back and breast of the male have rounded tips and are indistinguishable from the corresponding feathers of the female. Ground color and lacing of all the feathers is similar to that in the female.

Two varieties are known, the Silver and the Golden, for both of which the above description holds. The only difference in the two

[1] The experimental work for this report was practically completed while the writer was working in the Department of Zoölogy of the University of Chicago. Funds for the work were provided by the Sex Research Committee of the National Research Council, administered by Professor F. R. Lillie. The writer is indebted to Professor Lillie, not only for the financial aid, but even more for the suggestion of the problem and for constant criticism and encouragement.

varieties is the ground color of the plumage. The one is a smooth silver, and the other is a light brown. Every feather in both cases is laced, or at least tipped, with black. The general tone of the color among the birds of either variety may differ considerably by reason of the variations in the width of the black lacing. In poorly bred birds some of the feathers may be almost entirely black, or practically without lacing.

The perfect Sebright male is the result of the most careful breeding. In run-down flocks birds will appear showing feathers which are distinctly cocky, and the two median tail feathers (sickles) will be elongated. Were it not for the high standard insisted upon by the fanciers, it seems probable that the males would soon be ordinarily cock-feathered. Pézard, Sand, and Caridroit (1924*a*) speak of a standard cock-feathered strain of Sebrights in France.

Not only the results of experimental investigation but also the very fact of the occurrence of henny feathering in cocks make it apparent that certain of the characters sexually dimorphic in fowl are entirely distinct in their reactions from other characters also sexually dimorphic. Comb, wattles, and ear lobes, collectively referred to as "head-furnishings," together with voice and behavior, fall into one category, and plumage into another. Although the reactions of the spurs correspond to those of the plumage for the most part, the fact that birds with henny feathering may possess well-developed spurs seems to demand a special category for them. The full development of the first group of characters is directly dependent upon the presence of the testis. On the other hand, the plumage attains its greatest development in the complete absence of the gonads, and is restricted by the presence of either gonad. Inasmuch as it has been found that both the male and the female, when subjected to gonadectomy, tend to develop a "capon type" of plumage, one may say, without concerning himself with the nature of the phenomena, that, in breeds that exhibit sex dimorphism of plumage, the inhibition is greater in the case of the ovary than of the testis. The difference in the nature of this inhibition is the basis for the plumage dimorphism. The development of the spurs is apparently also inhibited by either gonad, and ordinarily to a greater degree by the ovary than by the testis.

## PLATE I

FIG. 1.—Normal Sebright cock. The position is poor, but the picture is given to show the "square" tail (cf. Fig. 2).

FIG. 2.—Normal Sebright cock in which the two median tail feathers are elongated.

FIG. 3.—Typical Sebright capon (No. 43, Table I).

FIG. 4.—Sebright capon with ovary implanted (No. 41, Table I); (*a*) a group of henny neck feathers among the hackles of the capon; (*b*) henny wing coverts among cocky feathers; (*c*) henny back feathers among cocky feathers.

## PLATE II

FIG. 5.—Normal Sebright pullet.

FIG. 6.—Ovariectomized Sebright pullet (No. 70, Table II).

FIG. 7.—Ovariectomized Sebright pullet in which testis was found growing on the right side of the abdominal cavity (No. 74, Table II).

FIG. 8.—Ovariectomized Sebright pullet which was found to contain two testicular masses and an ovotestis (No. 40, Table II).

PLATE I

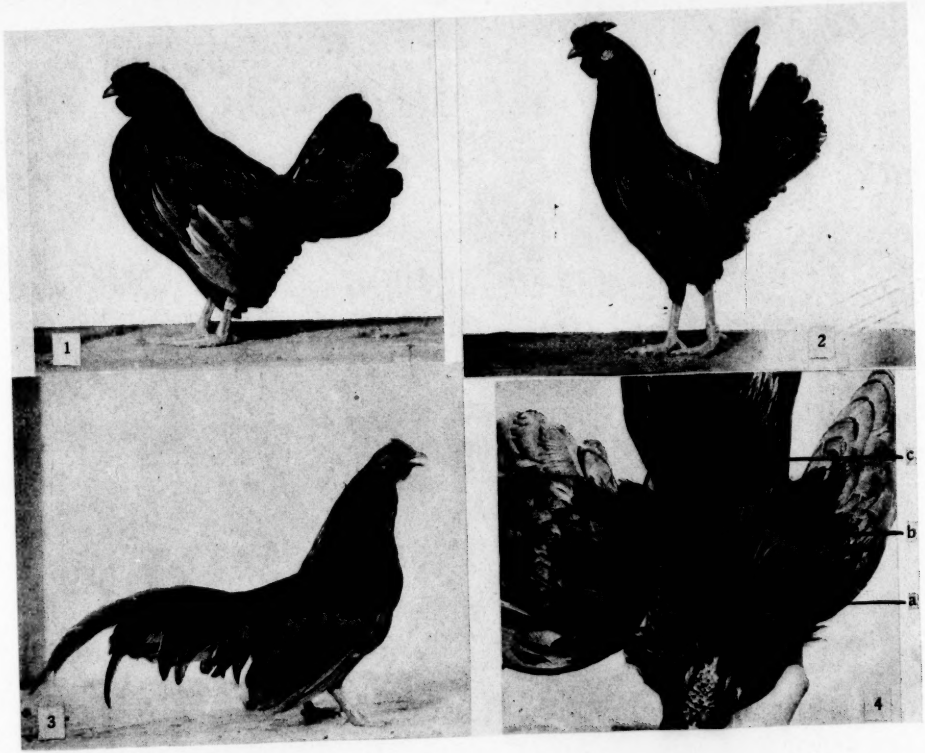
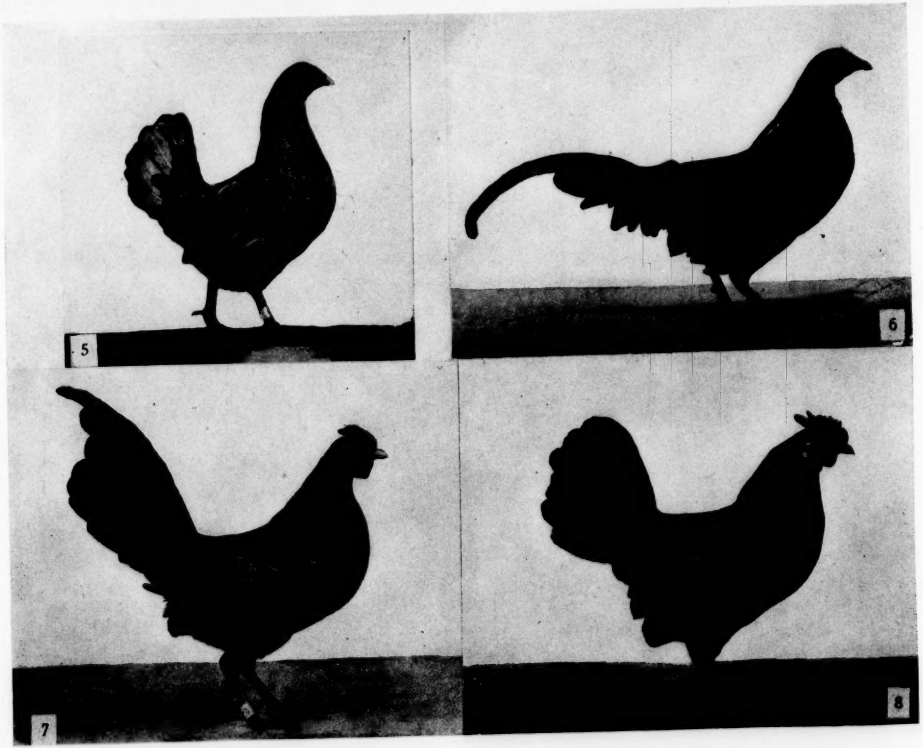


PLATE II



Attention has been called by a number of writers to the difference in the relations of the various characters to the gonads. Goodale (1916*a*) recognizes the independent reactions of the head-furnishings, voice and behavior, spurs, and the plumage. Zawadowsky (1922) refers to cock-feathering and spurs as "independent" characters, and head-furnishings, instinct, voice, and hen-feathering as "dependent" characters, the former being developed completely in spite of the removal of the testis or as a result of the removal of the ovary, the latter disappearing upon the removal of the gonad and reappearing upon the regeneration or implantation of the gonad. Pézard (1918) noted that castration altered certain of the sex characters but not others.

The two chief contrasting groups of sex characters must be dealt with separately. This report treats especially of the significance of plumage dimorphism, although the reactions of the other characters must necessarily be referred to correlatively.

## MATERIAL AND METHODS

All of the birds used in the experiments to be described were of the Sebright bantam race, and all but three were of the Golden variety.[1] The three exceptions were Silver. They need no special mention since they did not appear to differ in any way in their reactions.

Operations, principally gonadectomy, were performed at ages varying from a few weeks to over a year. Birds were prepared for the operation by isolating them and depriving them of food for 2 days. This insured an empty intestine which would fall clear of the site of the operation when the abdominal cavity was opened. The incision into the abdominal cavity was made between the last two ribs, in length from 3 to 5 cm., depending upon the size of the bird. A spring "spreader" such as is used in commercial caponization separates the ribs so that the working space may be 2 or 3 cm. wide. In view of the large size of the gonad that was sometimes removed (the testes of Sebrights are relatively much larger than those of other breeds,

[1] Most of the birds used for these experiments were purchased as very young birds from Mr. Valentine Petzold, of Elkhart, Indiana, who was known to breed only from first-class stock. Some were hatched from eggs obtained from Mr. Harry Thorne, of Chicago, whose breeders were also known to be prize-winning stock.

especially in the younger birds), this opening is small enough to lend some difficulty to the operation. Testes 2 cm. by 3 cm. have been removed, which, of course, entirely conceal their own mesorchium except under tension. Once the gonad is available, it is stripped of its covering peritoneum and freed from the underlying tissue by careful manipulation with two pairs of small forceps, aided by such clamps or retractors as are convenient or applicable. This step is dangerous because the gonad is adherent to the thin ventral wall of the large iliac vein where the latter is embedded in the kidney shortly posterior to the junction of the two iliac veins. A slight tear in this vein renders the procedure very difficult by reason of the gushing of blood that occurs when the weakened place is stretched, and anything more than a very slight tear is almost certain to result fatally. It has not been found practicable to occlude this vein below the site of the operation. After the gonad is lifted out, the exposed site is cauterized to destroy any strands of gonadial tissue that might have been overlooked. The operation is completed by binding the separated ribs together with one or two ligatures and closing the skin incision by continuous suture. Without undue loss of blood the bird recovers immediately and seeks food. It was found safer, in the case of males, to remove but one gonad at a time, performing the second operation from the opposite side at least 2 weeks after the first.

The testes were ordinarily removed intact. Only from the youngest females was it possible to remove the ovary intact. As the bird grows older, the ovary becomes quite diffuse, so that it was expedient to snip off small portions as they were freed from the body wall.

Excised gonads were immersed in Bouin's fluid at body temperature and fixed for 24 hours before being allowed to cool. In the case of the older birds, the testes were incised to allow more rapid penetration of the fixing fluid.

Measurements of the head-furnishings and spurs were taken at the time of the operation and at intervals thereafter. It was considered desirable to photograph each bird before performing an operation. This was done as far as possible; but since the photographing was done out-of-doors, the weather conditions made regularity impossible.

To have a basis for the recognition of altered plumage, a few feathers from critical areas were plucked and filed in envelopes at the time of the operation. Representative feathers were always taken from the dorsal part of the neck (where hackles were destined to appear in the capon), the back, about on a line with the ends of the long wing coverts, the saddle region, and the tail proper (rectrices). In a great many cases, primary and secondary flight feathers (remiges) were also taken, as well as lesser wing coverts, scapulars, and tail coverts.

If the plumage showed a condition of particular interest at the time of the death of the bird, the head and legs were severed from the body and preserved, and the entire skin was removed with as little damage to the plumage as possible and prepared with arsenic.

Ovarian implantation in capons was accomplished by opening simultaneously the body cavities of a young female and the capon. The ovary was then removed from the female and without delay placed in the body of the capon. Several pieces were implanted lest one might not "take." These were put in small pockets among the muscles of the body wall and under the peritoneum. Several pieces were also thrown loose in the body cavity. Grafts were found to have grown in all these places.

## RESULTS

### 1. GENERAL

*a*) *Orchectomy* (*Table I*).—The first operations performed were those of simple gonadectomy of cocks. Eleven birds were obtained which, at least for a time, showed all the characteristics of capons; and three more, which died or were killed before the change was complete, showed partial transition (Table I). My results agree in general with those of Morgan (1915, 1917, 1919, 1920*a*); viz., a luxuriant plumage appeared (described in detail in the next section), the head-furnishings were reduced or remained small, and the spurs continued their growth (Plate I, Fig. 3). The behavior of the birds was that described for capons of ordinary breeds.

The first indication of successful caponization is given by the head-furnishings, which become pale and shrink very rapidly. Replacement feathers appear in the areas deplumed at the time of the

operation, and by the fourth week these are sufficiently advanced to permit recognition of their cocky condition.

From the fact that replacement feathers in areas deplumed within 24 hours after the removal of the testes are cocky even at their very tips, it is apparent that the continuity of the supply of hormone is very important. The period of duration of testicular effect after the removal of the testes must be very short, but the exact determination of the time will depend upon a study of the length of time occupied by repair processes in the follicle from which

TABLE I

| Bird No. | Plumage Resulting from Orchectomy | Final Plumage | Time Elapsed between Orchectomy and Last Examination | Post-Mortem Findings | Remarks |
|---|---|---|---|---|---|
| 73.... | Mixture | Mixture | 1 mo. | ..... | Lost to rats |
| 77.... | Mixture | Mixture | 3 mos., 3 wks. | No gonad | Died before molt was complete |
| 65.... | Mixture | Mixture | 4 mos., 1 wk. | No gonad | Died before molt was complete |
| 61.... | Capon type | Capon type | 10 mos., 1 wk. | No gonad | Silver Sebright |
| 71.... | Capon type | Capon type | 10 mos., 2 wks. | No gonad | |
| 67.... | Capon type | Capon type | 11 mos. | No gonad | |
| 31.... | Capon type | Capon type | 11 mos., 2 wks. | No gonad | |
| 41.... | Capon type | Mixture | 15 mos., 2 wks. | Ovary | Ovary implanted after change to capon type was complete, 9 mos. before last examination |
| 43.... | Capon type | Mixture | 15 mos., 3 wks. | Ovary | Ovary implanted after change to capon type was complete, 10 mos. before last examination |
| 45.... | Capon type | Capon type | 16 mos. | Small testis | |
| 47.... | Capon type | Capon type | 17 mos. | Small testis | |
| 49.... | Capon type | Capon type | 17 mos. | No gonad (laparotomy) | Left intact for further experiments |
| 37.... | Capon type | Capon type | 22 mos. | Small testis | |
| 3.... | Capon type | Hen type | 25 mos. | Testis | |

the feather was torn and the time of the beginning of proliferation for the new feather.

Three cases (Nos. 3, 37, and 45) were observed in each of which a testis was found to have regenerated on the site of one of those previously excised. One of these birds (No. 3), killed 25 months after the operation, had quite completely reverted to the henny type of plumage. The other two, killed, respectively, 22 and 16 months after gonadectomy, were still capon-feathered. The regenerated testis of No. 3 was considerably larger than that of either of the other two, measuring approximately 8 mm. in length and 5 mm. in diameter. Some of its tubules, but not all, showed complete spermatogenesis, there being abundant spermatozoa in the lumina.

Post-mortem dissection of No. 37 revealed a small, whitish body, roughly spherical, about 2 mm. in diameter, attached at the site of the excised right gonad. This body contained a few tubules that could be recognized as seminiferous tubules, though no spermatozoa were present. At one edge the sections cut across several coils of ductuli efferentes. On the left side of the body cavity, a small white patch appeared on the exposed surface of the adrenal. Section proved it to be a thin cap of tissue containing testicular tubules. Spermatozoa were not present. In addition to these two growths, there were several whitish areas upon the wall of the small intestine. Pieces of the intestinal wall were cut out to include these areas, and section showed a few testicular tubules to be present. These growths must have been due to accidental autotransplantation during the operation for the removal of the testes.

In the case of No. 45, a growth measuring 7 mm. by 4 mm. was removed from the left side, at the site proper for a gonad. This mass was only partially organized into characteristic tubules, and no spermatozoa were present.

In none of the several growths removed from the body cavity of No. 37, nor in their total, was the amount of testicular tissue comparable to the amount removed in one piece from No. 3. This fact is probably to be correlated with the condition of the plumage. This seems to imply one of two alternatives: either the testicular tissue is present in insufficient quantity to affect the plumage, or the influence of the gonad has not been acting for a sufficient length of time to cause any recognizable modification of the feathers. Since, however, the effect of the withdrawal of the gonadial secretion is recognizable in the feather within a month of the time of removal of the gonad, and since neither in No. 37 nor in No. 45 do any of the newly erupted feathers appear henny, the difference between No. 3 and Nos. 37 and 45 is more probably due to a quantity difference than a time difference, except in so far as time is a factor secondarily in accounting for mass differences.

Since No. 3 lived 25 months after gonadectomy, and No. 37 only 3 months less (and since No. 3 was fully hen-feathered, whereas No. 37 showed no indications of hen-feathering), there must have been either some difference in the length of time which elapsed after the

operation before the testis began to regenerate or some difference in the rate at which regeneration took place. It is impossible to say, from the present data, which condition is the more probable or why either such differential should exist.

One cannot fail to recognize an effect of the regenerating gonad upon the head-furnishings. In the case of No. 3, the head-furnishings at the time of death were larger than before the operation, although the bird was mature (18 months old) when operated upon. In the cases of Nos. 37 and 45, the head-furnishings at the time of death were, although not equal to those of mature intact cocks, by no means those of capons. They were about two-thirds full size, erect, and of a good red color. Although this suggests a fractional influence of the gonad, there is more than a possibility that the submaximal size merely indicates that the death of the bird interrupted a growth that would have run to completion if given time enough. The question can, of course, not be answered by an experiment in which the mass of testicular tissue is an increasing quantity.

Numbers 41 and 43 were castrated and allowed to assume good capon plumage. Then, 7 and 6 months, respectively, after castration, several pieces of young ovary were implanted in the abdominal cavity and body wall of each. Neither of these birds molted after the ovarian implantation, so neither of them assumed again a full henny plumage; but deplumed areas became hen-feathered, and feathers which were occasionally lost were replaced by those of henny type. Under the influence of the ovary the head-furnishings enlarged somewhat, but not to full male size. These birds were heard to crow, and their behavior among other birds was frequently that of a cock. This anomalous reaction has been noted by Goodale (1917).

*b*) *Ovariectomy* (*Table II*).—The results of ovariectomy are complex. In the first place, the operation is more difficult than orchidectomy, due to the flat attachment of the gonad. The operation becomes increasingly difficult as the bird matures; and after laying has begun, it is practically impossible of accomplishment. Therefore in a number of cases a small amount of ovarian tissue was retained in the bird and the operation was without effect. In other cases cocky feathers appeared in the deplumed areas not different

from the replacement feathers of gonadectomized cocks. With the exception of two cases (to be described below), however, such birds did not acquire a completed plumage of the capon type. A reversion to the henny type occurred, leaving the bird until the next molt with

TABLE II

| Bird No. | Plumage Resulting from Ovariectomy | Final Plumage | Time Elapsed between Ovariectomy and Last Examination | Post-Mortem Findings | Remarks |
|---|---|---|---|---|---|
| 90... | Mixture | Mixture | 2 mos. | Right: testis | |
| 60... | Mixture | Mixture | 3 mos., 3 wks. | Right: testis (few tubules)<br>Left: ovary (ovo-testis?) | |
| 98... | No record | Hen type | 4 mos., 3 wks. | Right: testis<br>Left: testis | |
| 100... | Hen type | Hen type | 5 mos., 2 wks. | Right: testis<br>Left: ovary | Ovarian tissue retained through operation—degenerating |
| 64... | Mixture | Hen type | 6 mos. | Left: ovary | |
| 74... | Mixture | Mixture | 6 mos. | Right: testis | |
| 80... | Mixture | Hen type | 6 mos. | ..... | Lost to rats |
| 12... | Mixture | Mixture | 6 mos., 1 wk. | Left: small testis | |
| 102... | Mixture | Mixture | 7 mos., 1 wk. | Right: small testis<br>Left: testis (?) | |
| 104... | Mixture | Mixture | 7 mos., 1 wk. | Right: testis | |
| 106... | Mixture | Mixture | 7 mos., 1 wk. | Right: testis (?) | |
| 108... | Mixture | Mixture | 7 mos., 1 wk. | Right: testis<br>Left: testis | |
| 110... | Mixture | Mixture | 7 mos., 1 wk. | Right: testis<br>Left: testis | |
| 114... | Mixture | Mixture | 7 mos., 1 wk. | Right: testis | |
| 70... | Capon type | Capon type | 9 mos. | Right: testis<br>Left: testis (?) | |
| 68... | Mixture | Hen type | 11 mos. | Right: testis<br>Left: testis | |
| 58... | Mixture | Hen type | 11 mos., 2 wks. | Right: testis<br>Left: small testis | |
| 54... | Mixture | Hen type | 12 mos. | Left: testis | |
| 18... | Mixture | Hen type | 13 mos. | Right: testis<br>Left: testis | |
| 50... | Mixture | Hen type | 13 mos., 2 wks. | Right: testis<br>Left: testis | |
| 92... | Capon type | Capon type | 14 mos., 3 wks. | Right: testis<br>Left: small testis(?) | Mass on right side large but containing but few tubules |
| 40... | No record | Hen type | 16 mos., 2 wks. | Right: testis<br>Left: testis and ovo-testis | |
| 26... | Mixture | Hen type | 18 mos. | ..... | Lost to rats |
| 66... | Mixture | Hen type | 18 mos., 3 wks. | Right: testis | |

a plumage partly henny and partly (in the deplumed areas) cocky (Plate II, Fig. 7).

The reason for this reversion was apparent when the body cavities were opened and there were found, invariably, new gonads, either ovary or testis or both. Since in the Sebright the testis serves to maintain the plumage of the cock in the henny condition, it is not surprising that the development of a testis in the ovariectomized hen caused the plumage to be of the henny type.

Nineteen cases were found in which a testis had developed on the right side of the body cavity, on the site proper for a gonad (Table II). Such organs have been found on the right side of ovariectomized females in ordinary breeds of fowl by Benoit (1923*a* and 1923*b*), Domm (1924), Finlay (1925), and Zawadowsky (1926). Goodale (1916*b*) and Zawadowsky (1922) observed growths on the right side but did not identify them as testes.

Eleven of the foregoing cases showed also a testis on the left side, in place of the excised ovary. Three showed either an ovotestis or an ovary on the left side. Two cases, not included in any of the above categories, showed a testis only on the left side.

It must be pointed out that since the plumage is henny in the presence of either the testis or the ovary, the plumage reaction in those birds which developed both testicular and ovarian tissue following ovariectomy may not be assigned to either. The significant facts demonstrated by the operations upon the females are that the plumage produced in the absence of the gonad is similar to that produced by the male in the absence of the gonad, and that the plumage again develops as the henny type upon the development of a new gonad.

Bird No. 64 is a case of simple regeneration of the retained fragments of the excised ovary, and gives a history of having produced, for a short time after the operation, feathers of the cocky type. At the time of its death, feathers of the henny type were again being produced, although some of the cocky feathers were still retained in the plumage.

Cases Nos. 66, 74, 104, 106, and 114, as well as those several cases in which there developed a testis on the site of the excised ovary, showed that entirely similar reactions take place with respect to plumage upon the development of testis as upon development of ovary.

That the testes which develop in the ovariectomized females are comparable to the testes of males is further demonstrated by the fact that the head-furnishings in birds which have acquired such testes become slowly cock-like. Figure 7 of Plate II shows an ovariectomized pullet (No. 74) 6 months after the operation. Cocky feathers may be noted in various regions, but henny feathers were

being produced when the bird was killed. Testis was found, but no ovary. Figure 8 of Plate II shows a bird (No. 40) 14 months after ovariectomy, in which the head-furnishings are well developed. This bird crowed occasionally. Its body contained a small ovotestis on the left side, and two testicular masses, one on either side on the proper sites.

In two instances (Nos. 70 and 92) ovariectomized pullets assumed quite completely the capon type of plumage, and retained it until 9 and 14 months, respectively, after the operation. Number 70 (Plate II, Fig. 6), running in the flock, was for some time assumed to be a capon, but upon closer examination, its number revealed its original sex. The record is substantiated by the preserved ovary which bears the corresponding number. The sex of No. 92 is also confirmed by the preserved ovary.

The plumage of both of these birds is somewhat darker than that of the capons. This darkness is due to the fact that the black lacing of the feathers, usually restricted in the capons to the feather web, extends nearly or actually to the tip of the feather, involving the whole or part of the fringe. Some of the feathers have the reddish brown color characteristic of the capon feather.

Upon dissection, the abdominal cavity of No. 70 was found to contain on the right side a small testis, approximately 8.5 mm. in length and 2.4 mm. in diameter, rounded anteriorly and tapering to a point posteriorly. On the left side there was no gonadial tissue at the proper site; but a small, round nodule, 2 mm. in diameter, was removed from the mesentery of the ileum which resembled testis, although the identification could not be made with certainty.

The abdominal cavity of No. 92 contained, on the right side, a fairly large growth (5 mm. in diameter) on the gonad site, but in only a very thin cap over the growth were tubules present which could be identified as testicular. On the left side there was a very small growth (1.5 mm. by 4 mm.) which was testicular, if gonadial at all.

How are we to account for the failure of these two birds to produce again a henny plumage upon the appearance of the testicular growths? There are two plausible explanations, neither of which can be established without the examination of further similar cases.

It is possible, as the first suggestion, that the gonads found in each case were slow in starting development, so that they became effective only such a short while before the birds were killed that the effect upon the plumage could not yet be observed. By reason of the previously mentioned darkness of the feathers, it is difficult to determine whether the younger, growing feathers were henny or cocky in their proximal portions; and, of course, those feathers which were already full grown would be retained until the next molt, which might be some months away.

The second possibility is that both of these birds were of such constitution as to be cock-feathered even in the presence of the testis. Such birds are well known among Sebright males, and it is significant that the two most cocky-feathered males in the writer's flock were darker than the capon. Number 70 was, before the operation, a well-laced bird, but not strikingly dark. Number 92 was unusually heavily laced prior to the operation. Number 70 was purchased from Mr. Petzold, whose Sebright stock was known to be excellent and as free as possible from the tendency toward cock-feathering. Number 92, however, was purchased from a huckster, so that the stock was not known at all, and was, in all probability, poor.

Consistent with this interpretation is the fact that in the case of No. 92, the bird had developed head-furnishings much greater than those of true capons. Number 70, on the contrary, never showed a greater development of head-furnishings than that characteristic of capons.

The only previous record of the acquirement of cocky feathering by the Sebright hen is found in Tegetmeier's *Poultry Book* (London, 1873). This author described a hen with a diseased ovary, which became cocky in respect to plumage.

With respect to the effect of the ovary in an orchectomized male, it suffices to say that the plumage returns to the henny type. The effect is very prompt, feathers plucked at the time of the operation being replaced by feathers of the henny type. Figure 4 of Plate I shows such hen-feathered areas among the hackles of the neck, among the wing-coverts, and among the back feathers of the capon.

### 2. THE ALTERATIONS IN THE VARIOUS TRACTS

The plumage transformation[1] affects the entire bird, at least some feathers of every tract being altered. In the normal Sebright cock, the feathers of the spinal pteryla are of one type over the entire back. This is a blunt, black-laced, fairly short feather with a light-brown ground color. The feathers over the neck are somewhat different in that they have a fringe surrounding the feather outside of the black lacing, either black or light-brown in color (Plate III, Fig. 21). In this fringe, the barbs are provided with barbules only on their proximal sides, or are practically barbule-free. This fringe is found also in the case of females.

The hackles which appear in the capon form a broad cape extending well over the sides of the neck. They are very long, acuminate, and golden-brown in color, with an occasional black tip, and with a wide fringe of barbs entirely free from barbules (Plate III, Fig. 22). The most lateral are much shorter than those of the mid-dorsal region. A few posterior feathers of the neck are tipped with black, except that the fringe of barbule-free golden-brown barbs extends completely around the end. Under these, and only in the mid-dorsal region, are a very few feathers which are striped with black for their entire length, the two stripes converging to meet distally. Though some of these may be quite long, none are as long as the longest of the more anterior hackles. They are broader in proportion to their length, and taper rather abruptly to a sharp point. The last feathers of this group are the shortest, and differ from the corresponding feathers of the cock chiefly in being more pointed. The ground color is the ordinary light brown. Some of the most lateral feathers of the neck region of the capon may be of a reddish brown color instead of the more general golden brown.

Posterior to the neck feathers the type occurs which is characteristic for the entire back proper (Plate III, Figs. 17 and 19). In the capon, the distal third of the feather is bordered by a fringe of barbule-free barbs, usually golden brown in color, though a greater or lesser amount of black may appear (Plate III, Figs. 18 and 20). The web

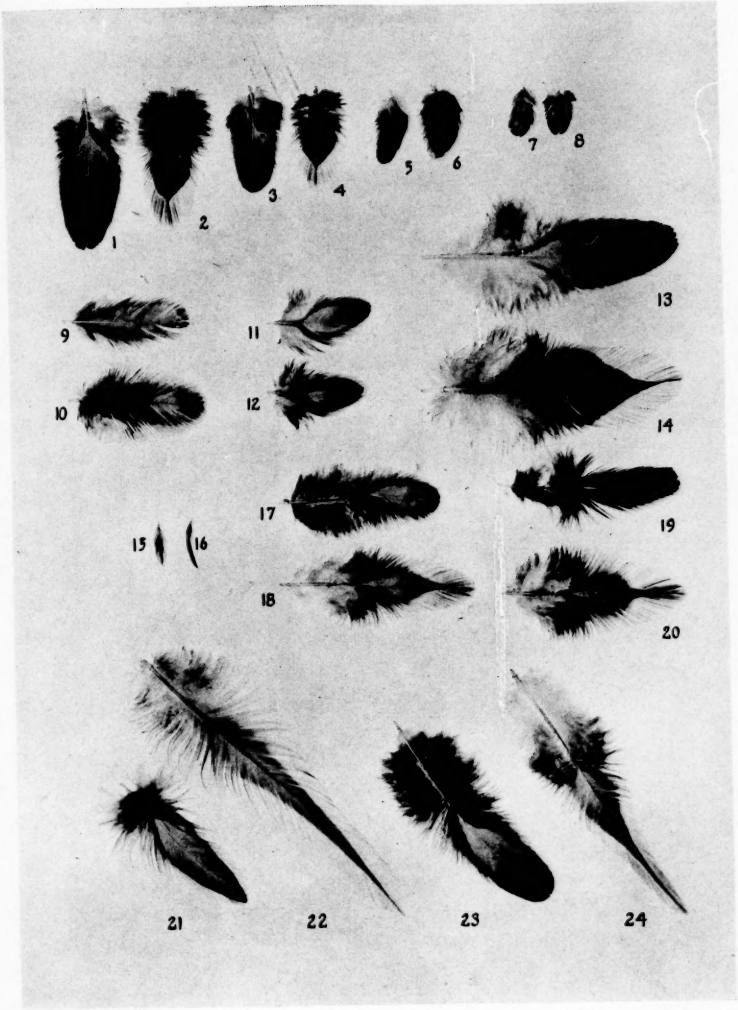
[1] This "transformation" involves the loss of the original feather and the development of a completely new one, except that feathers which are growing at the time of the operation may continue their growth in a new pattern (*vide infra*, p. 311).

## PLATE III

(Feathers of the capon compared with feathers of the normal Sebright cock. Reduced about one-half.)

FIG. 1.—Lesser secondary wing covert, normal cock.
FIG. 2.—Lesser secondary wing covert, capon.
FIG. 3.—Lesser secondary wing covert, normal cock.
FIG. 4.—Lesser secondary wing covert, capon.
FIG. 5.—Lesser secondary wing covert, normal cock.
FIG. 6.—Lesser secondary wing covert, capon.
FIG. 7.—Lesser primary wing covert, normal cock.
FIG. 8.—Lesser primary wing covert, capon.
FIG. 9.—Axillary, normal cock.
FIG. 10.—Axillary, capon.
FIG. 11.—Short scapular, normal cock.
FIG. 12.—Short scapular, capon.
FIG. 13.—Long scapular, normal cock.
FIG. 14.—Long scapular, capon.
FIG. 15.—Capital, normal cock.
FIG. 16.—Capital, capon.
FIG. 17.—Back, normal cock.
FIG. 18.—Back, capon.
FIG. 19.—Back, normal cock.
FIG. 20.—Back, capon.
FIG. 21.—Dorsal neck, normal cock.
FIG. 22.—Dorsal neck, capon.
FIG. 23.—Saddle, normal cock.
FIG. 24.—Saddle hackle, capon.

PLATE III



proper enclosed by the fringe may or may not be bordered with a black stripe, which, when present, by the convergence of the two sides, gives the feather an exceedingly sharp appearance. Toward the posterior part of the back, in front of the tail coverts, the color of the barbs outside of the black stripe becomes redder, and there is often a black tip.

The spinal tract laterally gives rise to the saddle feathers, overhanging the "saddle" of the bird, immediately behind the legs. In the cock, these are not different from the back feathers (Plate III, Fig. 23). In the capon, though not as long as the longest of the neck hackles, they are distinct hackles (Plate III, Fig. 24). In their distal third they are even narrower than the neck hackles, because they consist of but a shaft with short, barbule-free barbs. They are redder than the neck hackles, similar to the reddish brown of the back. The proximal third is fluffy. The middle third shows a web of the ordinary light brown color, striped irregularly with black.

Posterior to the saddle feathers proper, there are transition feathers between the saddles and the tail coverts. In the cock this means only a gradual increase in length in successive feathers. In the capon, the transition feathers are not only intermediate in size, but also in form and pattern. The black stripes extend farther and farther distally, reducing the proximal extent of the free barbs until the tips of the feathers are quite blunt. Proximally the black stripe drops out, so that none of the feathers is completely laced.

The tail coverts are in the caudal pteryla, and the dorsal set is roughly divisible into two series, a shorter group anteriorly and a longer group posteriorly. In the cock, even the longer coverts are not much more than half as long as the true tail feathers (Plate IV, Fig. 4). They show the usual lacing. In the capon, on the other hand, the tail coverts are greatly elongated, those of the longer series curving above and beyond the tail feathers proper (Plate IV, Figs. 5 and 6). The webs show a light brown color, and are tipped, but not always laced, with black.

The rectrices (tail feathers proper) of the cock are fairly short, quite straight, and with "square" ends (Plate IV, Fig. 1). The web is light brown and laced with black. The rectrices of the capon are much longer and usually have less perfect lacing (Plate IV,

## PLATE IV

Feathers of the capon compared with feathers of the normal Sebright cock. Reduced about one-half.)

FIG. 1.—Rectrix, normal cock.

FIG. 2.—Rectrix, capon.

FIG. 3.—Sickle, capon.

FIG. 4.—Tail covert, normal cock.

FIG. 5.—Tail covert, capon.

FIG. 6.—Tail covert, capon.

PLATE IV

Fig. 2). In some, the black border is confined to the tip, while in others the lacing continues with interruptions for the length of the web. The two median tail feathers show the greatest elongation, being sometimes over twice the normal length (Plate IV, Fig. 3). It will be remembered that these median feathers are sometimes extra long in the cocks (Plate I, Fig. 2).

Though the color of the web of the tail coverts and rectrices cannot be said to be changed as in the case of the hackles and back feathers, the brown color may be of noticeably darker shade, and the general tone is darkened by the occurrence of black splashes and stippling in the web.

Coverts ventral to the tail feathers correspond to the dorsal tail coverts, except that in the capon none show the extreme elongation seen in the case of the dorsal coverts, and the feathers are laced (Plate V, Figs. 7 and 8).

Anteriorly the spinal tract is continuous with the capital tract, embracing the small feathers of the back of the head and at the sides of the comb. These in the cock are similar to the feathers of the dorsal side of the neck, greatly reduced in size (Plate III, Fig. 15). The black lacing may be incomplete or absent. In the capon, the feathers are reddish-brown, without lacing, longer, narrower, and sharply pointed (Plate III, Fig. 16).

The ventral tract extends from the neck immediately behind the mandible to the vent. The most anterior feathers in the cock are very short, blunt, and light brown in color, with a poorly defined lacing (Plate V, Fig. 3). The edges are ragged, owing to a fringe such as was seen in the dorsal neck feathers. In the capon, in the distal half of the feather there is a broad fringe of barbs entirely free of barbules, lustrous reddish brown in color (Plate V, Fig. 4). The web is mostly or entirely black. The feathers are of about the same size in the cock and the capon, but they are sharply pointed in the capon.

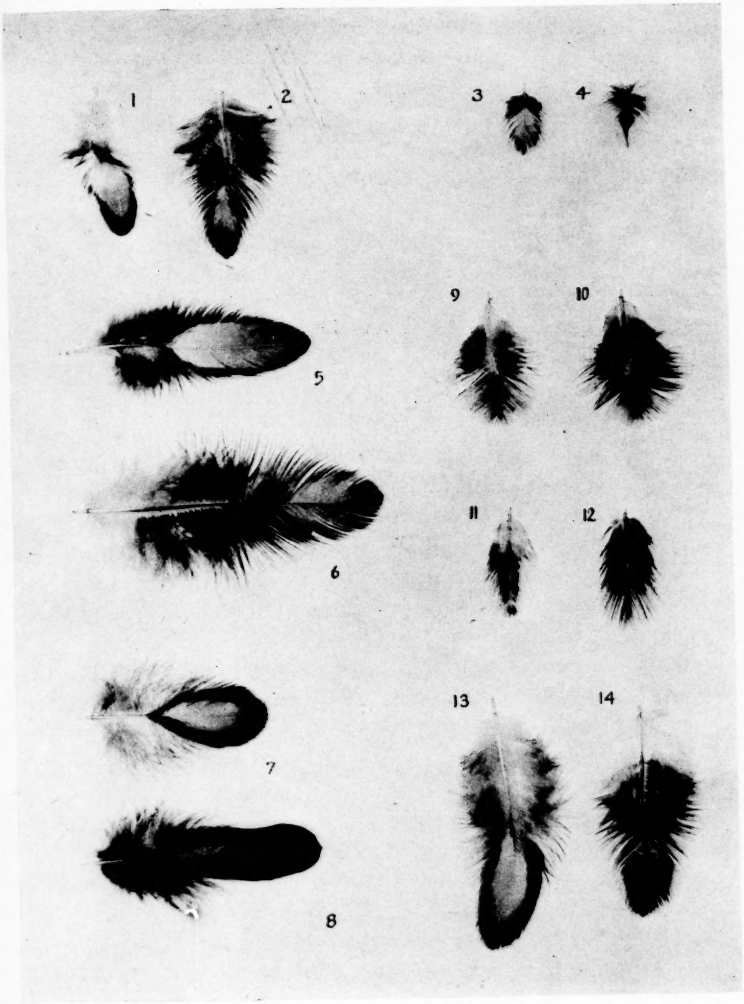
Feathers over the crop are blunt, light brown, and laced with black in the cock (Plate V, Fig. 1). The barbs have barbules for their entire length. The same feathers in the capon are broader and more pointed (Plate V, Fig. 2). In the distal third of the feather, each barb is for a short distance at its end free from barbs on the distal side. The color is not greatly different, though the web and shaft of the capon feather are darker.

## PLATE V

Feathers of the capon compared with feathers of the normal Sebright cock. Reduced about one-half.)

FIG. 1.—Crop, normal cock.
FIG. 2.—Crop, capon.
FIG. 3.—Ventral neck, normal cock.
FIG. 4.—Ventral neck, capon.
FIG. 5.—Breast, normal cock.
FIG. 6.—Breast, capon.
FIG. 7.—Ventral tail covert, normal cock.
FIG. 8.—Ventral tail covert, capon.
FIG. 9.—Abdominal, normal cock.
FIG. 10.—Abdominal, capon.
FIG. 11.—Crural, normal cock.
FIG. 12.—Crural, capon.
FIG. 13.—Femoral, normal cock.
FIG. 14.—Femoral, capon.

## PLATE V



The breast feathers are much the same as the crop feathers in the cock and the capon, respectively. The feathers are successively longer, passing posteriorly (Plate V, Figs. 5 and 6).

The feathers posterior to the breast, covering the ventral wall of the abdomen, are fluffy; but even these tend to develop more luxuriantly in the capon (Plate V, Figs. 9 and 10). The general tone of the feather is darker, the darkness of the shaft being particularly noticeable.

The feathers of the wing constitute two pterylae: the humeral and the alar. Those of the humeral tract lie, when the wing is closed, in a narrow strip covering approximately the scapular region, and are known as the scapular feathers, though they grow from around the humerus. All other feathers of the wing are alar.

The scapular feathers are quite short anteriorly and are successively longer toward the posterior end of the tract. In form and pattern the feathers of the cock are quite similar to the feathers of the spinal tract, into which the humeral tract blends medially (Plate III, Figs. 11 and 13). In the capon, the shortest of the scapulars are but slightly modified (Plate III, Fig. 12). A narrow fringe of barbs from which the barbules are lacking on the distal side extends beyond the lacing. The feathers are perceptibly pointed. A slight fringe on the cock feather is due to the fact that at the ends of the barbs the barbules of the distal sides are shorter than the barbules of the proximal sides, so that the barbules of adjacent barbs do not meet.

The longer scapular feathers are markedly altered (Plate III, Fig. 14). The black stripes converge immediately upon reaching the maximum breadth of the web, so that a large diamond-shaped area is inclosed. The black stripes may be very thin distally. The outline of the feather is similar in the cock and the capon, because in the capon reddish-brown barbs, free from barbules, extend beyond the black stripe to fill the same area as is occupied by web in the case of the cock. The web proper of the capon feather and the shaft are darker than those of the cock.

The alar feathers are arranged in rows over and under the wing. The longest of the wing feathers are the primary remiges or flight feathers, growing on the pinion. These in the cock are light brown in color and are usually not laced, but there is a black border a centi-

## PLATE VI

Feathers of the capon compared with feathers of the normal Sebright cock. Reduced about one-half.)

FIG. 1.—Primary remix, normal cock.

FIG. 2.—Primary remix, capon.

FIG. 3.—Secondary remix, normal cock.

FIG. 4.—Secondary remix, capon.

FIG. 5.—Greater primary wing covert, normal cock.

FIG. 6.—Greater primary wing covert, capon.

FIG. 7.—Greater secondary wing covert, normal cock.

FIG. 8.—Greater secondary wing covert, capon.

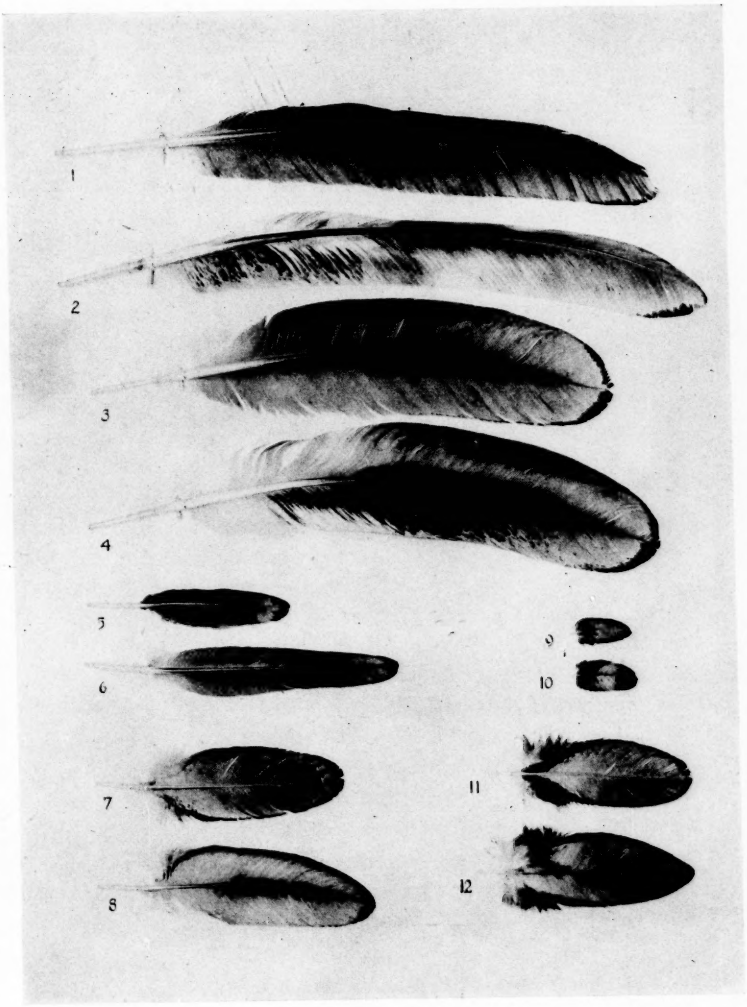
FIG. 9.—Intermediate primary wing covert, normal cock.

FIG. 10.—Intermediate primary wing covert, capon.

FIG. 11.—Intermediate secondary wing covert, normal cock.

FIG. 12.—Intermediate secondary wing covert, capon.

## PLATE VI



meter or two in length near the distal end of the medial side of the feather, and the tip may be black (Plate VI, Fig. 1). In the capon these feathers show an increased length but the pattern is not markedly altered (Plate VI, Fig. 2).

The secondary remiges also show an increased length in the capon. In the cock they are usually completely laced, though the lacing may be very fine (Plate VI, Fig. 3). In the capon, the black may appear only at the tip of the feather or it may extend as a border part way down the sides (Plate VI, Fig. 4).

Overlying the bases of the primary and secondary flight feathers on the dorsal side, there occurs a row of greater coverts which mimic quite closely the flight feathers in both the cock and the capon, save that they are much smaller (Plate VI, Figs. 5 and 6). The increase in length of the greater coverts of the primary remiges of the capon is, however, much greater in proportion to their original length than the increase in the case of the remiges themselves. In the case of the greater coverts of the secondary flight feathers, the increase over the cock size is less, but some of them tend to become more pointed at the tip (Plate VI, Figs. 7 and 8). Color changes are similar to those described for the remiges.

The intermediate coverts of the primary remiges overlying dorsally the bases of the greater coverts are, in the cock, short, blunt feathers with black tips, if not completely laced (Plate VI, Fig. 9). Color is not regularly altered. The tip is black in the capon, but a few of the most distal barbs may be reddish brown (Plate VI, Fig. 10). The shaft is darker in the capon, the difference being particularly noticeable on the under side of the feather. A very narrow fringe occurs around the tip of the capon feather, in which the distal sides of the barbs are free from barbules.

The intermediate coverts of the secondary remiges of the capon show less blunt ends than those of the cock (Plate VI, Figs. 11 and 12). In addition the web is shorter and out of proportion, more of the shaft being occupied by fluff proximally. Color changes are not marked; but the web is somewhat richer brown in the capon, and the shaft is darker.

The lesser coverts, in several irregular rows overlying the bases of the intermediate coverts, are very small in the case of those re-

lated to the primary remiges. Those of the capon are of richer color than those of the cock, and the shaft is darker (Plate III, Figs. 7 and 8). The lesser coverts in relation to the secondary remiges are markedly altered in the posterior rows, though toward the anterior border of the wing the alterations are comparatively slight. In the case of the most posterior rows, the coverts of the capon (Plate III, Figs. 2 and 4) are not regularly longer or shorter than those of the cock (Plate III, Figs. 1 and 3), but the web is reduced to a diamond by the fact that the barbs of the distal portion of the feather are free from barbules. The free barbs are reddish brown. The black lacing of the cock feather is represented in the capon by an irregular stripe at the sides of the diamond. The ground color of the capon feather is richer, and the shaft is reddish brown. These feathers are very similar to those found on the back, in both the cock and the capon, respectively. The lesser coverts at the anterior margin of the wing of the capon (Plate III, Fig. 6) show a very narrow fringe of barbs, free of barbules on their distal sides, not found in the corresponding feathers of the cock(Plate III, Fig. 5), and the shaft is redder.

On the ventral side of the wing occur a few axillary feathers, corresponding in position about to the scapulars on the dorsal side. The feather and the individual barbs are longer in the capon (Plate III, Fig. 10), the shafts are redder, and the ground color is darker than in the cock (Plate III, Fig. 9).

The arrangement of the coverts on the ventral side of the wing is similar to that on the dorsal side, the feathers being, however, quite sparse and small. The alterations produced by caponization are not so marked as those described for the dorsal coverts, and will not be described in detail here. One common difference is that the web of the capon feather is less compact than that of the cock feather. Quite commonly, also, though not universally, the shafts of the feathers of the capon are redder than those of the cock.

Feathers of the femoral tract of the cock are longer and wider than those of the back, to which they are otherwise similar (Plate V, Fig. 13). The femoral feathers of the capon differ in that the web and shaft are redder and there is a narrow fringe of barbs which have barbules only on their proximal sides (Plate V, Fig. 14).

Feathers from the shank (crural pteryla) have a loose web, and the color pattern is not regular (Plate V, Fig. 11). Those of the capon are larger, tend to have more black, and the shaft is redder (Plate V, Fig. 12).

An item of plumage dimorphism that is not distributed with sufficient regularity to warrant specific mention is the occurrence of a brilliant greenish sheen of the black lacing. In the normal Sebright, the black lacing may show a slight luster, more common in the cock than in the hen. The brilliance of the capon sheen is not approximated in the normal female, however, and only in small areas in the cock. In the capon, although it does not appear in the rectrices or remiges, almost any other feather that has a sufficient amount of black at its distal end shows the greenish luster. The black fluff and the black web next to the shaft proximally is always dull. The largest lustrous areas are found on the tail coverts, both dorsal and ventral. Other good examples may be found on back feathers and wing coverts.

### 3. THE EFFECT OF OVARIECTOMY ON PLUMAGE

Every attempt to produce a permanently gonadless bird from a female has been frustrated because the bird sooner or later developed a testis. The operative removal of the ovary gives a temporarily gonadless condition, the effect of which is seen in such feathers as are developed before a new gonad becomes functional. From the condition of the feathers that grow in the areas deplumed at the time of the operation and from the appearance of two birds that underwent molts shortly after being ovariectomized (Nos. 70 and 92), it is evident that the feathers of the female in the absence of the gonad will develop after the manner of the corresponding feathers of the castrated cock (Plate II, Fig. 6).

Plate VII illustrates a number of capon-type feathers that were obtained from No. 70, compared with corresponding feathers from normal hens.

The neck feathers of the poulard are hackles, having a fringe of barbule-free barbs, golden brown in color, as in the capon (Plate VII, Figs. 11 and 12).

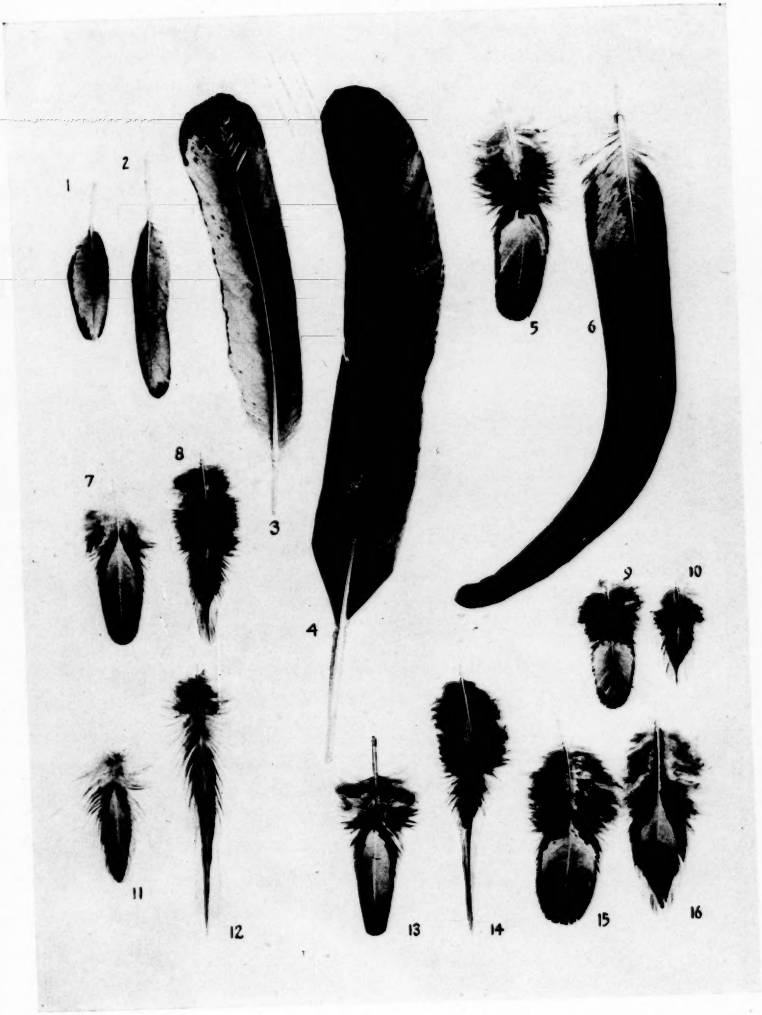
Back feathers of the poulard also show the fringe of the barbule-

## PLATE VII

(Feathers of the poulard [No. 70] compared with feathers of the normal Sebright hen. Reduced about one-half.)

FIG. 1.—Greater primary wing covert, normal hen.
FIG. 2.—Greater primary wing covert, poulard.
FIG. 3.—Rectrix, normal hen.
FIG. 4.—Rectrix, poulard.
FIG. 5.—Tail covert, normal hen.
FIG. 6.—Tail covert, poulard.
FIG. 7.—Back, normal hen.
FIG. 8.—Back, poulard.
FIG. 9.—Lesser secondary wing covert, normal hen.
FIG. 10.—Lesser secondary wing covert, poulard.
FIG. 11.—Dorsal neck, normal hen.
FIG. 12.—Neck hackle, poulard.
FIG. 13.—Saddle, normal hen.
FIG. 14.—Saddle hackle, poulard.
FIG. 15.—Long scapular, normal hen.
FIG. 16.—Long scapular, poulard.

## PLATE VII



free barbs (Plate VII, Figs. 7 and 8). The amount of reddish-brown coloration is apt to be less than in the feathers of the capon, but the redder of the poulard feathers show less black than the blacker of the capon feathers.

Hackles appear over the saddle of the poulard comparable in form and color to the saddle hackles of the capon (Plate VII, Figs. 13 and 14).

The elongation of the dorsal tail coverts is no less striking in the poulard than in the capon (Plate VII, Figs. 5 and 6). The same darkening of the ground color occurs, and the lacing exhibits the green sheen.

Figures 3 and 4 of Plate VII show that the tail feathers proper of the poulard are enlarged as in the capon. No sickle feather is shown, but one may be seen in Figure 6 of Plate II.

Scapular feathers, in the humeral pteryla, show the same difference between hen and poulard as between cock and capon (Plate VII, Figs. 15 and 16), except that there is more black mixed in with the reddish brown of the free barbs.

The elongation of the greater converts of the primary remiges is equivalent in the poulard and the capon (Plate VII, Figs. 1 and 2).

As striking as the elongation of the greater coverts of the primary remiges is the alteration of the lesser coverts of the secondary remiges. Figures 9 and 10 of Plate VII show that the changes from the hen feather to the poulard feather are comparable to the changes from cock feather to capon feather.

Other changes less marked but equally significant can be recognized in the poulard as being similar to the changes in the capon, as, for instance, the slightly elongated remiges, the acuminate capital feathers, the more pointed crop feathers, and larger and more lustrous ventral tail coverts. The brilliant green sheen of the lacing is displayed on various parts of the body.

#### 4. THE EFFECT OF THE GONAD UPON THE GROWING FEATHER

So far we have dealt with two plumage types as being in a sense allelomorphic; the one, henny, occurring in the presence of the gonad; and the other, cocky (capon type), in the absence of the gonad. The question now arises, What of the feather that is in the process

of differentiation at the time the influence of the gonad is withdrawn, or imposed after a period of absence? As a matter of fact, it is possible for a feather to start its development toward one type and then change and continue toward the other type.

However distinctive the color changes may be, it is difficult in the Sebright to recognize on a basis of color a change from henny to cocky feather, or vice versa, because of the fact that the feather is not uniformly colored. In the proximal portion of even the capon feathers, the web is invariably light brown, and the black regions are dull. Although the distinctive golden brown and reddish brown colors are confined to the barbule-free barbs, such barbs are not restricted to those colors, and it is very common for portions of them to be black. There is no regularity about the distribution of the black and the brown if both are present. Sometimes the line between the black and brown will follow sharply along one barb, or it may cut at an angle across several barbs.

The most striking structural difference between henny and cocky feathers is the occurrence in the cocky feathers of barbs which at least for a considerable portion of their length are free from barbules. These are found in the hackle feathers of the neck and saddle, back feathers, scapular feathers, and lesser wing coverts. They are not found anywhere in either the normal cock or normal hen, except in the neck feathers, where barbs may occur which are free from barbules in a small portion of their length. Except in these neck feathers, all the barbs of all the feathers of the hen and cock are provided with barbules in their entire length. No capon feathers have all of their barbs barbule-free. A line following the limits of the barbules of successive barbs is usually a slight curve, with the convexity toward the shaft, each barb being provided with barbules for a greater portion of its length than the barb distal to it. This line is descernible in the feather shown in Plate III, Figure 14. But the transition is not a direct one from the barbule free condition to that of ful barbs. Between the barbule-free area and the fully barbuled area there is a narrow strip in which there are barbules on the proximal side of each barb only. A portion of this strip can be seen in Figur 20 of P late III.

The facts of barbule distribution, together with the facts of

color distribution, seem to indicate the existence in the capon feather of a proximo-distal, and a medio-lateral gradient of gonadial effect. This is of significance in connection with transition feathers.

None of the transition feathers in my collection show a purely hen-type portion succeeded immediately by a purely capon-type portion. Morgan (1919) describes transition feathers of which "the outer end .... shows the original or normal shape and color, while the inner end shows the new characteristics." He says later:

> It will be noticed that the change after castration involves the color, shape, and the presence and absence of barbules in those parts of the bird that are peculiar in that respect. The transition in these characters is quite sharp—as sharp, in fact, as is compatible with the passage from one structure to that of an entirely different kind without any discontinuity of growth.

Morgan's transition feathers were collected from cocks changing to capons. The writer's collection includes some such, and some from birds changing capon type to hen type. Although it is questionable just how sharp a transition may be and still be compatible with the passage of one structure to that of an entirely different kind without any discontinuity of growth, the transition feathers are by no means as regular as Morgan's description would lead one to expect.

A group of transitional lesser wing coverts from a cock changing to a capon (No. 71) are particularly interesting. The first of them has a black-laced rounded tip, and the barbs at the end of the feather are provided with barbules to their very ends. This much of the feather is normal for the cock (henny) feather. The black tip shows a greenish luster, however, such as could not be expected in a henny feather. Thus it appears that a capon feature is superposed upon a henny feature, either by simultaneous or successive processes. Behind the rounded tip, the edges of the compact web run about parallel, while the actual width of the feather is increasing, owing to the fact that the barbs are free of barbules in their distal portions, the length of the free portion increasing passing proximally. At about the usual distance from the end of the feather the demarcation line turns lateralward and the feather does not differ from the typical capon feather.

The second feather of the series also has a black-laced tip, but be-

yond the compact portion of the web, and for a greater distance on one side than on the other, there extend short ends of the barbs that are barbule-free or provided here and there with barbules only on their proximal sides. Again the black lacing shows the green luster of the capon, and again the sides of the compact web are parallel for a short distance before curving outward as in the capon.

The third feather of the series differs from the second in that the free barbs beyond the black-laced rounded tip are longer, and especially so on one side of the shaft. Furthermore, a very few barbs off the very end of the shaft are reddish brown, as in the capon.

In the fourth feather the black tip is much reduced in size. There are short, free barbs extending beyond the tip, among which a few are reddish brown. The web proximal to the black tip, however, instead of remaining at least as wide as the tip, narrows abruptly, the boundary making a line at right angles to the shaft. There occur barbs in this region, therefore, which are free of barbules at their distal ends, then provided with barbules in their middle portions, and then again barbule-free for a short distance, and finally again provided with barbules where they form part of the compact web next to the shaft. Proximal to this region the feather is typically capon type.

The fifth feather is bilaterally markedly asymmetrical. The one side is laced continuously with black, distally lustrous and becoming gradually dull proximally. Beyond the lacing is a fairly wide fringe of free barbs, though not so wide as in the capon. The other side of this feather is of the typical capon type.

The transition feathers from gonadectomized birds that are returning from the capon type to the hen type by reason of the growth of a new gonad are difficult to recognize because of the fact, to which attention has previously been called, that the feathers are distinctively capon type in structure only in their distal portions, even in the pure capon. Only certain indubitable transitions will be described here.

The first to warrant description are two saddle feathers drawn from a cock (No. 3) which, having been castrated, assumed the full plumage of a capon, but which, after some months, again assumed the plumage of a cock (henny). It was found that a testis had de-

veloped on the site of one of the excised testes. Both of these feathers are about the length and shape of the normal cock feathers of the bird. They are laced with black completely, and the web color is that of the cock's normal feather. The first of them shows, however, extending beyond the lacing, a very narrow fringe of barbs which are not provided with barbules on both sides. In the saddle feathers of this bird as an unoperated cock, every barb was provided with barbules on both sides to its very end. In the feather in question, the first eight or ten barbs on either side of the tip of the shaft are barbule-free beyond the lacing, after which the next hundred or so have barbules on the proximal side only. The second of these two saddle feathers is like the first except that it has no barbs at all that are barbule-free. The entire fringe is made up of barbs that lack barbules on their distal sides but have them proximally. A number of feathers similar to this one were found.

A group of feathers showing similar features were drawn from the back of a bird (No. 54) which, having had its ovary removed, developed a testis on the right side. These feathers are of practically normal shape for henny back feathers. At the tip of each feather, instead of there being barbs after the type of either the capon or the hen, there are barbs which bear barbules in their outer portions only on their proximal sides.

From the saddle of the same bird, there were drawn feathers of the same order as those just described. They are normal hen type in shape and color, except that the lacing is not continuous around the tip. The barbs at the tip of the feather lack distal barbules in their outer portions.

Considered alone, the group of irregular feathers taken from the cock that was changing to capon might be satisfactorily accounted for on the simple assumption that such features of the feather as were henny were those features that were already fixed in type when the influence of the gonad was abruptly withdrawn. This is, indeed, probably very nearly the case. However, in the case of the feathers of the capon changing to cock, it cannot be assumed that the feathers were simply interrupted in the course of a specific evolution toward a capon type, for if such were the case, those parts of the feather developed early in point of time would be of capon type, just as, in the

transitional feathers of the reciprocal change, the parts first developed were of henny type. The point of conflict is that certain of the peculiarities found in the transitional feathers of the bird changing from cock to capon were similar to those in the bird changing from capon to cock. Apparently another factor enters: this factor is the ability of the various elements of the feather to respond to different strengths of the gonadial hormone. This seems to imply that the effect of the gonad is fractionable. Additional evidence of this lies in the correlation of the fact that in birds which were changing from the capon type to the hen type the gonad was increasing very slowly in size, at least at first, whereas in the opposite case the entire gonad was removed suddenly, with the fact that the intermediate (transitional) condition is more pronounced in the former case than the latter. Although consistent with the findings of Morgan (1919), Souba (1923), and Finlay (1925) with respect to the relation between the degree of development of the comb and the size of the testis, such an interpretation is not strictly in accord with the "all-or-none law" of the action of the bird's testis propounded by Pézard (1920, 1921, 1922*a*, and 1922*b*).

Applied to the cases at hand, this would mean that certain elements of the feather were dependent upon a greater strength of the gonadial hormone for their development than other elements, namely, certain barbules, and certain of these more than others. Moreover, a general proximo-distal and medio-lateral gradient has been described for the whole feather. When the gonad is suddenly removed from the body as in operative castration, there is a rapid falling off of the strength of the hormone. Certain elements of the growing feather are already fixed in the hen type. Among other elements, the distal barbules of the barbs near the tip of the feather are the first to lose their developmental capacities, followed by the proximal barbules of the same barbs and the distal barbules of the next barbs proximally, and so on to the limit of the alterations. This wave passes very rapidly in the case of the castrated cock because of the rapidity with which the hormonic strength falls off. In the case of the change from the capon type to the hen type of feather, however, the restoration of the strength of the hormone is a gradual matter. Therefore the wave is more distinct. Those

elements which are able to complete their development toward the hen type with but slight hormonic influence become henny first: namely, the proximal barbules and even some of the distal barbules of the more proximal barbs. This leaves the barbs free on their distal sides for a considerable distance from the tip of the feather, and at the very tip there may still be some barbs which are not yet affected. Growing later, these few barbs would have gotten their proximal barbules, and still later, the development of distal barbules would have been complete also.

This conception involves two factors, namely, that of the order of fixation of growing parts of the feather and that of the response of the various parts to unequal strengths of hormone of the gonad. Transition forms can thus be explained which cannot be fitted to a theory based only on the matter of the presence or absence of the gonad.

Goodale (1916*b*) described transition feathers, some of which he explained on the basis of the time of differentiation of parts, and others on the basis of a difference in the susceptibility of certain parts to hormones.

## DISCUSSION

Morgan (1919), who first experimentally castrated the Sebright, reasoned that, since the testis of the hen-feathered male apparently exerted the same influence upon feathering as did the ovary, the testis must be in some respect hermaphroditic; i.e., it must contain secreting cells ordinarily restricted to the ovary. Boring and Morgan (1918) identified certain cells of the Sebright testis as identical with the "luteal" cells of the ovary (Pearl and Boring, 1918) and gave them the same name. These cells were said to be absent from the testis of the adults of ordinary cocks. Hence the theory was proposed that hen-feathering was due to the secretion of "luteal" cells, and that the special condition of the Sebright was due to the presence of "luteal" cells in the testis as well as in the ovary. Boring and Morgan's identification was made on histological grounds, however, and is open to question. Pease (1922) has shown that the "luteal" cells occur in the testes of cocks of ordinary breeds as well as in the testes of Sebrights, and, further, that they are not present in any breed except prior to the onset of spermatogenesis. Pease suggests a

trophic function for these cells. He denies, then, that the "luteal" cells are confined to hen-feathered cocks and that they are of endocrine nature.

Examination of the testes of the fowl by Goodale and Nonidez (1924), and of the testes of various wild breeds by Yocom (1923 and 1924), by Morgan (1923), by Yocom and Phillips (1924), and by Goodale (1924), has shown it to be quite impossible to correlate the presence of "luteal" cells with hen-feathering. Greenwood (1925) reports that "luteal" cells in testis grafts are not correlated with hen-feathering.

Nonidez (1922), confirmed by Fell (1924) for the case of the ovary, determined that the cells in question were not "luteal" cells at all, but remnants of degenerating sexual cords, and therefore readily to be homologized in the testis and the ovary. Nonidez described the cells only for the immature gonad, however, and found them in the testes of Leghorn chicks as well as Sebright. Furthermore, he offers the opinion:

> From a purely histological standpoint, the deep regressive changes undergone by these seminiferous tubules in which fat-laden cells arise, strongly suggest that the formation of the clusters in the testis is an abnormal feature due to regressive differentiation of immature elements rather than to progressive changes leading to the formation of new morphological units.

Later Nonidez (1924) confirmed Pease in finding that the remnants of the sex cords are absent in the testes of adult hen-feathered cocks. As a further obstacle to Morgan's hypothesis, he offers data from two cases of hypo-genitalism. These were cockerels of a cock-feathered breed in which the male plumage was well developed. In the malnourished testes, however, sex-cord remnants ("luteal" cells of Boring and Morgan) appeared in abundance.

It is scarcely possible to draw a conclusion from such conflicting evidence other than that, at the present time, there is no sound histological basis for the "endocrine hermaphrodite" theory of hen-feathering. Histological support of such a theory would demand demonstration of an unusual course of development and the consequent presence of a group of cells in the adult Sebright testis not ordinarily testicular constituents. This would have to be shown, not only for the normal Sebright testis but also for the testes which

have regenerated in males after castration and which have appeared in females after ovariectomy. It is to be presumed that the source of the ovarian hormone lies in the cortex of the ovary, which is derived from a second generation of ingrowths from the germinal epithelium. No evidence has been presented that such ingrowths occur in the testes of hen-feathered cocks.

Perhaps the strongest experimental support for Morgan's theory has come from the work of Zawadowsky (1922) and Pézard, Sand, and Caridroit (1925), and Finlay (1925), who have produced artificial hermaphrodites by the implantation of ovary into cocks. With respect to plumage in such birds, the ovarian influence predominates; and hence they are hen-feathered. However, other male characters, such as the head-furnishings, persist in the male condition as in the case of the Sebright.

This "endocrine hermaphrodite" theory, although it has been much disputed, has remained until recently the most attractive theory of hen-feathering. Seemingly conclusive evidence against such an explanation, however, appears in the work of Roxas (1926). Roxas found that a castrated Sebright male became hen-feathered again under the influence of an implanted Leghorn testis and that the plumage of a castrated Leghorn did not become henny under the influence of an implanted Sebright testis. This removes entirely the probability of there being any significant difference between the testes of the ordinary breeds of fowl and the testes of Sebrights.

In another place Morgan (1920*b*) says:

> It is also interesting to note that the removal of the testes of cock-feathered birds causes in them also some slight changes in the same secondary sexual characters in which they differ from the hen-feathered birds. . . . It appears, then, that even in the cock-feathered birds there is some inhibition to the growth of these feathers produced in the normal testis. This inhibition may be supposed to go so far in the hen-feathered races as to completely stop down the male plumage to that of the hen.

This suggestion of Morgan's, with its original implications, has never received much consideration, although Benoit (1924) stated the proposition again. The idea appears in a new light since the work of Roxas, the emphasis being placed on the reaction of the feathers rather than on the action of the testes. There is an inhibitive

action on the part of the testes of both ordinary and hen-feathered cocks; but the inhibitive action of the Sebright testes does not appear to be any more potent than that of the Leghorn testis. The difference between the two breeds, then, lies in the susceptibility of the feather germs to the gonadial hormones—a difference founded on the genetic constitution of the birds.

Buchanan (1926), on the basis of the examination of the thyroid glands of Sebrights and of one abnormally hen-feathered Silver-grey Dorking, suggests that the phenomenon of hen-feathering among males is due to the underfunctioning of the thyroid gland, any condition of the plumage representing the algebraic sum of the influence of the thyroid and the gonad, the two glands being antagonistic. Though subjected to a different interpretation, the situation reported is not inconsistent with the data of Horning and Torrey (1922) bearing on the results of the feeding of thyroid substance to fowl. The work of Crew (1923 and 1925), of Crew and Huxley (1923), and of Cole and Reid (1924), also indicates an influence of the thyroid gland upon the plumage.

It may be said that all feathers tend to develop toward the capon type and that interference is being effected when the feather stops its development in any other form. It must be remembered that the ordinary course of development involves the successive appearance of three markedly different forms—the down feather, the juvenile feather, and the cock feather. Since the actual feather is non-living, these forms are indicative of the condition of the feather germ during the periods of differentiation of the respective feathers. Feathers are normally produced at certain periods in the evolution of the feather germ. If the normal evolution of the germ be upset, as it may by the withdrawal of the sex hormone supply, a feather type other than that normal for the period will be produced. If a physiological condition can be produced which is not ordinarily met with in nature, a feather type should be expected which is intermediate between the natural types or extreme in some direction. That is what is accomplished when the effect of the gonad is fractionated, as, for instance, by the slow growth of the gonad following ovariectomy. Feathers of apparently intermediate condition have just been described, and Pézard, Sand, and Caridroit (1924*b*) have ob-

served pigmentary modifications following ovariectomy that were different from simple secondary sexual modifications. The change, then, between ordinary cock and capon feathering, and the change between Sebright normal male and capon feathering, although morphologically markedly different are not necessarily expressions of qualitatively different physiological alterations but may be quantitative changes in each instance. It is thus possible to consider the Sebright testis as the qualitative equivalent of the ordinary cock testis, and its relation to feather development as being qualitatively similar. The findings of Roxas are entirely consistent with this view. Pézard (1924), however, does not consider that the testicular inhibition as seen in the Sebright is comparable to the inhibition of the testes of ordinary breeds.

## SUMMARY

Male and female Sebrights were gonadectomized, and it was observed that the plumage reactions to the operation were quite comparable, both sexes tending to produce a type called "capon plumage." The production of a complete capon plumage is interrupted in either sex in case of the acquirement of either testicular or ovarian tissue by implantation or by regeneration or by compensatory growth of the rudimentary right gonad in the female, under which circumstances the plumage again becomes of the hen type. It appears, therefore, that the plumage of the Sebright is a very labile character and that the two types (capon and hen) may be made to appear in any individual regardless of its original sex, and, furthermore, that the same gonadial tissue in the soma of either sex brings about the production of the same plumage type.

There is some evidence that intermediate types of feathers can be produced. This is not consistent with the "all-or-none law" and suggests the fractionability of gonadial effect.

Support is lent to the view that the testis of the Sebright is the qualitative equivalent of the testis of cocks of ordinary breeds in regard to hormone production, the difference of plumage type being attributable to a difference in the reactiveness of the feather germs rather than to the glandular complex of the testis.

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